DOCTOR OF MEDICINE

Dapagliflozin Regresses Left Ventricular Hypertrophy in People with Type 2 Diabetes

Brown, Alexander

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Abbreviations

ABPM	Ambulatory Blood Pressure Measurement
ACE	Angiotensin Converting Enzyme
ACEi	Angiotensin Converting Enzyme Inhibitor
aCMQ	Automated Cardiac Motion Quantification
ADA	American Diabetes Association
AGE	Advanced Glycation End Products
ARB	Angiotensin Receptor Blocker
ASE	American Society of Echocardiography
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BP	Blood Pressure
BPV	Blood Pressure Variability
BSA	Body Surface Area
CAD	Coronary Artery Disease
CI	Confidence Interval
CMD	Coronary Microvascular Dysfunction
CO	Cardiac Output
CV	Cardiovascular
CVD	Cardiovascular Disease
CMR	Cardiovascular Magnetic Resonance
CMRI	Cardiac Magnetic Resonance Imaging
CNR	Contrast To Noise Ratio
CRF	Case Record Form
DT	Deceleration Time
E/A Ratio	Early to Late Diastolic Ratio
ECG	Electrocardiogram
Echo	Echocardiogram
EDV	End Diastolic Volume
EDWT	End Diastolic Wall Thickness

EF	Ejection Fraction
ELISA	Enzyme Linked Immunosorbant Assay
ERK 1/2	Extracellular Signal Regulated Protein Kinase 1 and 2
ESV	End Systolic Volume
FDA	Food and Drug Administration
FGRE	Fast Gradient Echo Sequence
FIRI	Fasting Insulin Resistance Index
FLASH	Fast Low Angle Shot
FPG	Fasting Plasma Glucose
GFR	Glomerular Filtration Rate
GLS	Global Longitudinal Strain
GLUT 1	Glucose Transporter 1
GWAS	Genome Wide Association Studies
$HbA1_c$	Glycated haemoglobin
HCM	Hypertrophic Cardiomyopathy
HFpEF	Heart Failure with Preserved Ejection Fraction
HFrEF	Heart Failure with Reduced Ejection Fraction
HHF	Hospitalisation with Heart Failure
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOPE	Heart Outcomes Prevention Evaluation
HR	Hazard Ratio
hsCRP	High Sensitive C-Reactive Protein
ITTA	Intention to Treat Analysis
IVRT	Isovolumetric Relaxation Time
LIFE	Losartan Intervention for Endpoint Reduction in Hypertension
LV	Left Ventricular
LVEF	Left Ventricular Ejection Fraction
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
LVMI	Left Ventricular Mass Indexed

LVSD	Left Ventricular Systolic Dysfunction
MAPK	Mitogen Activated Protein Kinases
MI	Myocardial Infarction
MMPs	Matrix Metalloproteinases
MPT	Mitochondrial Permeability Transition
MPO	Myeloperoxidase
MR	Magnetic Resonance
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NH	Nutritional Hygienic
NHE-1	Na^+/H^+ exchanger
NHS	National Health Service
NO	Nitric Oxide
NLRP-3	Nucleotide Binding Oligomerization Domain-Like Protein 3
NT-proBNP	N-Terminal Pro Natriuretic B-Type Natriuretic Peptide
OGTT	Oral glucose tolerance test
PIIIPP	Procollagen N-terminal Propeptide III peptide
PKC	Protein Kinase C
PPAR α	Peroxisome Proliferator-Activated Receptor α
RAAS	Renin Angiotensin Aldosterone System
ROS	Reactive Oxidative Species
RR	Relative Risk
RyR2	Ryanodine Receptors
SCAT	Subcutaneous Adipose Tissue
SDRN	Scottish Diabetes Research Network
SERC-2A	Sarcoplasmic Reticulum Ca^{2+}-ATPase
SGLT2	Sodium-Glucose-Co-Transporter 2
SNA	Sympathetic Nervous Activity
SNPs	Single nucleotide polymorphisms
SNR	Signal to Noise Ratio
SNS	Sympathetic Nervous System

SOD	Superoxide Dismutase
SPCRN	Scottish Primary Care Research Network
SSFP	Steady state free precession
SV	Stroke Volume
TCTU	Tayside Clinical Trials Unit
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
UK	United Kingdom
VAT	Visceral Adipose Tissue
WHO	World Health Organisation
XO	Xanthine Oxidase

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Declaration

I declare solely myself authored this thesis during my time as a Clinical Research Fellow at the University of Dundee. All data were collected and analysed by myself, including the statistics, apart from the abdominal MRI analysis that was performed by Stephen Gandy and blood analysis that was performed by Leslie MacFarlane. I was employed as a Clinical Research Fellow within the Department of Molecular and Clinical Medicine at the University of Dundee from November 2016 to April 2019.

I declare this work has not been submitted for a higher degree before. This work has been presented at national and international meetings as acknowledged within the thesis.

Signed: Alexander James Martin Brown Date: 11/05/2020

Thesis Summary

People with Type 2 diabetes (T2D) have a two to fourfold increased risk of cardiovascular mortality. Sodium–glucose cotransporter 2 inhibitors (SGLT2i) have been shown to reduce cardiovascular events, particularly heart failure, in cardiovascular outcome trials. One potential mechanism that may explain this benefit is regression of left ventricular hypertrophy (LVH); an independent predictor of cardiovascular events including incident heart failure.

We tested the hypothesis that dapagliflozin may regress LVH in people with T2D.

We randomly assigned 66 people (mean age 67 +/- 7 years, 38 males) with T2D, LVH and controlled blood pressure to receive dapagliflozin 10mg once-daily or placebo for 12 months. Primary endpoint was change in absolute left ventricular mass (LVM), assessed by cardiac magnetic resonance imaging.

In the intention to treat analysis, dapagliflozin significantly reduced LVM compared to placebo with an absolute mean change of -2.82g (95% confidence interval (CI): -5.13 to -0.51, p= 0.018). Additional sensitivity analysis adjusting for baseline LVM, blood pressure, weight and angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) prescription showed the LVM change to remain statistically significant (mean change -2.87g (95% CI: -5.27 to -0.48, p=0.02)). Dapagliflozin induced LVM regression was greatest in those with above-median left ventricular mass indexed (LVMI) to body surface area (BSA) at baseline (mean change -3.88g (95% CI: -7.33 to -0.43, p=0.029)). Dapagliflozin significantly improved pre-specified secondary end points including ambulatory 24 hour systolic blood pressure -3.6 mmHg (95% CI: -6.4 to -0.8; p=0.012), nocturnal systolic blood pressure -4.4mmHg (95% CI: to – 7.9 to -0.8; p=0.017), body weight -3.8 kg (95% CI: -4.9 to 2.6 p<0.001), visceral adipose tissue (VAT) -679.4 cm^3 (95% CI: -998.0 to -360.8, p<0.001), subcutaneous adipose tissue (SCAT) -609.8cm^3 (95% CI: -948.1 to -271.3 p=0.001), insulin resistance, HOMA-IR -2.6 (95% CI: -4.5 to -0.7, p=0.017), and high-sensitivity c-reactive protein (hsCRP) -1296.0 ng/l (95% CI: -2650.6 to -31.5, p=0.049).

Dapagliflozin treatment reduced LVM in people with T2D and LVH. This reduction in LVM was accompanied by reductions in systolic blood pressure, body weight, visceral and subcutaneous adipose tissue, insulin resistance and hsCRP. The regression of LVM suggests dapagliflozin can initiate reverse remodelling and changes in left ventricular structure which may be an important mechanism of action for the cardioprotective effects of SGLT2i.

Chapter 1 Introduction

1.1 Cardiovascular Disease in Diabetes

1.1.1 The Increasing Problem of Type 2 Diabetes

1.1.1.1 Definition and Diagnosis of Diabetes Mellitus

The World Health Organisation (WHO) in 2006 defined diabetes as: ‘a metabolic disorder of multiple aetiologies characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both’ (1). The American Diabetes Association’s (ADA) diagnostic criteria is based on one of four abnormalities: glycated haemoglobin ($HbA1_c$), fasting plasma glucose (FPG), random elevated glucose with symptoms, or abnormal oral glucose tolerance test (OGTT) as shown by Table 1(2).

1. HbA1c ≥6.5% (48mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.* **OR**
2. FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least eight hours.* **OR**
3. Two-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75-gram anhydrous glucose dissolved in water.* **OR**
4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

ADA: American Diabetes Association; HbA1C: glycated haemoglobin; NGSP: National Glycohemoglobin Standardization Program; DCCT: Diabetes Control and Complications Trial; FPG: fasting plasma glucose; OGTT: oral glucose tolerance test.

* In the absence of unequivocal hyperglycemia, criteria 1 to 3 should be confirmed by repeat testing.

Table 1 American Diabetes Association for the diagnosis of diabetes (2)

Type 2 diabetes Mellitus (T2D) is by far the most common type accounting for 90% of cases and is characterised by variable degrees of insulin deficiency and resistance. Type 1 diabetes Mellitus (T1D) accounts for only around 5-10% of diabetes in adults. It is characterised by autoimmune destruction of the pancreatic beta cells, leading to absolute insulin deficiency (2).

1.1.1.2 The Incidence and Burden of Diabetes Mellitus

Globally diabetes is becoming an increasing concern. In a global report on diabetes in 2016 the WHO stated that 422 million adults have diabetes (3). This is a substantial increase from 108 million in 1980 (3). The reasons for such an increase include an ageing population, increasing levels of obesity due to dietary excess and increasing levels of physical inactivity. Such an increase is a major health concern as diabetes is a major cause of blindness, renal failure and cardiovascular

disease (CVD). It is estimated that in 2015 1.6 million deaths were directly caused by diabetes and in 2030 the WHO believes it will be the seventh leading cause of death (3).

In the United Kingdom (UK) there are around 3.7 million people who have been diagnosed with diabetes. This does not include the estimated 1 million people with T2D who are undiagnosed (4). As a consequence, the cost of diabetes to the National Health Service (NHS) is huge. In total 14 billion pounds a year or over 1.5 million pounds an hour is spent on treating diabetes and its complications (5). This represents 10% of the NHS budget (5).

1.1.1.3 Diabetes and Cardiovascular Disease

It has long been established that patients with T2D have an increased risk of CVD with an increased risk of cardiovascular (CV) morbidity and mortality (6, 7). In the Framingham study having diabetes doubled the age-adjusted risk of CVD in men and tripled it in women (8). Diabetes remained an independent risk factor after adjusting for other atherogenic risk factors including hypertension, smoking and hypercholesterolaemia (8). In addition to diabetes being important as an independent risk factor, patients with diabetes have a larger burden of these other atherogenic risk factors (9). The addition of these risk factors is also known to increase the risk of CVD more steeply than for patients without diabetes (10). Patients with diabetes are therefore more likely to have multivessel coronary disease and at a younger age (11). Aggressive control of atherogenic risk factors are well established target areas for treatment of patients with diabetes to reduce the incidence of coronary events.

The mortality after cardiac events including sudden death is significantly increased compared to the non-diabetic population and diabetic patients are more likely to have complications associated with their myocardial infarction (MI) (9). Patients with diabetes have a four-fold increase of heart failure after an MI (12). Indeed, heart failure is another manifestation of diabetic heart disease. Men with diabetes are twice as likely to have heart failure as those without diabetes and women with diabetes have a five-fold increased risk (13).

Whilst diabetes commonly causes structural heart disease and heart failure via myocardial ischaemia and infarction, it can cause myocardial disease in the absence of major epicardial coronary artery disease(14). The term diabetic cardiomyopathy was first coined in 1972 by Rubler

et al and is defined as systolic of diastolic left ventricular dysfunction in people with diabetes mellitus without another obvious cause for this dysfunction such as hypertension, valvular heart or coronary artery disease. Rubler proposed this “new cardiomyopathy” having found at post mortem that 4 patients with diabetes had evidence of cardiomegaly/hypertrophy with congestive cardiac failure with no known cause (14). Imaging studies have demonstrated that LVH is an important feature of the diabetic heart and diabetic cardiomyopathy (15). Indeed, another manifestation of diabetes and cardiac disease and which is often overlooked is the increased prevalence of left ventricular hypertrophy (LVH) in patients with diabetes (16-18). LVH is a strong independent predictor for incident CV events and is at least as adverse as coronary artery disease (CAD) (19). LVH is discussed in more detail in the next section.

1.2 Left Ventricular Hypertrophy in Patients with Diabetes

1.2.1 Definitions and Diagnosis of Left Ventricular Hypertrophy

LVH is defined by an elevated left ventricular mass (LVM). This occurs due to an increase in the size of the cardiac myocytes in response to both haemodynamic and biomedical stress from either intrinsic or extrinsic sources. In response to pressure overload (e.g. hypertension) parallel addition of sarcomeres lead to an increase in myocyte width increasing LV wall thickness (concentric hypertrophy). With volume overload (e.g. aortic/mitral regurgitation) cardiac myocytes lengthen due to sarcomeric replication in series resulting in increased ventricular volumes (eccentric hypertrophy) (20). The precise mechanisms whereby LVH occurs are discussed in detail later.
In clinical practice the three main modalities used for diagnosing LVH are an electrocardiogram (ECG), echocardiogram (Echo) and cardiac magnetic resonance (CMR) imaging.

1.2.2 Electrocardiographic Assessment and Criteria for Left Ventricular Hypertrophy

In the past with the lack of imaging modalities to image the left ventricle meant ECG data were used as surrogate markers to assess for LVH. ECG criteria for LVH include the Sokolow-Lyon and Cornell voltage criteria. The Sokolow-Lyon criteria are defined as the sum of the S wave in V_1 and the highest R wave in either V_5 or V_6. LVH is diagnosed if the sum is greater than 35mm.

The Cornell voltage criteria are defined as the sum of the S wave in V_3 and the R wave in AVL. LVH is diagnosed if the sum is greater than 20mm in women and 28mm in men.

It is well recognised that these criteria whilst relatively specific, lack sensitivity in the diagnosis of LVH in comparison with Echo (21, 22). This is not surprising given the many factors which affect QRS voltage such as obesity, pulmonary disease, orientation of the heart and lead positioning (23). Whilst not sensitive the ECG does have prognostic significance. In the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) study, repolarisation abnormalities such as ST depression and T wave inversion on the baseline ECG along with voltage criteria for LVH were associated with a fourfold increase in the risk of heart failure related mortality (24). Despite this imaging techniques have now replaced the ECG for the assessment of LVH.

1.2.3 Echocardiographic Assessment and Criteria for Left Ventricular Hypertrophy

Echo has been used for over 30 years and has become one of the most important non-invasive imaging methods for evaluating cardiac morphology including LVM assessment (25). This is because it is a relatively cheap, rapid and a reasonably reproducible technique for measuring LVM. Studies have shown good correlation between echocardiographic assessments of LVM and post mortem findings in normal hearts (26). Indeed, many important studies examining the prognostic effects of LVH have used echocardiography (27-29).

To estimate LVM, 2D measurements of the left ventricle preferably over a number of cardiac cycles are made at end diastole which is defined as the frame following mitral valve closure (30). LVM is calculated from these measurements using the Devereux formula (31).

Historically body surface area (BSA) was the first anthropometric variable used to index LVM (32, 33). When using BSA the American society of Echocardiography (ASE) defines LVH as greater than $95g/m^2$ in women and $115g/m^2$ in men (30). However, with an increasingly obese population there are concerns that this underestimates the prevalence of obesity related LVH (34). Indexation to height to allometric exponent 2.7 has been shown to avoid missing LVH in obese patients and to be a better predictor for CVD outcomes (35, 36). The ASE recommends a cut off of $44g/m^{2.7}$ for women and $48g/m^{2.7}$ for men.

Whilst echocardiography is a specific, repeatable and far more sensitive measure of LVH in comparison to ECG it has a number of limitations. To obtain accurate measurements

echocardiography is dependent on a skilled operator to obtain adequate images and these may not be obtained in around a third of cases (37). Intraobserver measurements may vary about 5% between echocardiographic studies, while interobserver variability may reach 15% (25). Echocardiography also makes large geometric assumptions when calculating LVM and variations in ventricular geometry significantly effect calculated LVM (38).

1.2.4 Cardiac Magnetic Resonance Imaging for Left Ventricular Hypertrophy

CMR imaging in adults has become the gold standard for measuring LVM as it is highly accurate and reproducible (39). CMR provides high resolution images of the heart in any desired plane allowing 3D reconstruction of the heart with no geometric assumptions of the left ventricle required. Left ventricular (LV) short axis slices are obtained, and myocardial volumes are calculated by summing the endocardial areas for each slice and multiplying by the interslice-distance. LVM is calculated from the end-diastolic images by multiplying the myocardial volume by its specific density ($1.05 g/cm^3$). Selection of the correct basal slice is important as exclusion/inclusion of a slice can have a large effect on LVM and LVvolume measurements and is the greatest source of error. Papillary muscle mass has been shown to significantly affect LV volumes and mass (40). Papillary muscles should therefore be included in the LVM estimate and excluded from LV volume for greatest accuracy (41).

CMR assessment of LVM has been shown to be highly accurate and reproducible when compared to the actual heart mass determined at autopsy in both human and animal models (42, 43).

The reproducibility of LVM estimated by CMR has been demonstrated to be superior to Echo (38). A review of a number of human studies using CMR derived LVM measurements have showed a mean weighted interstudy variability of only 7.8g. The mean weighted intra- and interobserver variabilities were 4.8 and 9.0g, respectively. This was in contrast to the interstudy difference for M-mode and 2D Echo of 27.7g and 19.2g respectively (44-47). The improved reproducibility means CMR provides an ideal modality for assessing changes in LVM over time during therapeutic intervention.

The normal ranges of LVM derived from CMR depends on whether a traditional fast gradient Echo sequence (FGRE) (also known as fast low angle shot FLASH) or steady state free precession (SSFP) sequence is used. SSFP sequences generate a much higher contrast to noise ratio (CNR).

This is because contrast in balance SSFP is dependent on the T2/T1 ratio of the tissue imaged. Blood has a much higher T2/T1 ratio than myocardium and therefore blood appears much brighter. This allows excellent delineation of the myocardium from the blood pool and therefore more accurate LV mass assessment (48). SSFP is therefore now preferred over the older FGRE techniques which generally underestimates LV volumes and overestimates LVM due to its inferior border definition. (49)

A number of studies have published 'normal ranges' for data acquired on 1.5 Tesla systems. Common to all these studies is that LVM is significantly higher in men compared with women and in older age groups LVM reduces in men but remains constant in women(50-52). Table 2 shows the weighted mean "normal" values from references (50-52)

	Men			Women		
	$Mean_p$	SD_p	Lower/ upper limits*	$Mean_p$	SD_p	Lower/ upper limits*
EDV [ml]	160	27	106-214	132	23	86-178
EDV /BSA [ml/m^2]	81	12	57-105	76	10	56-96
ESV [ml]	54	14	26-82	44	11	22-66
ESV/BSA [ml/m^2]**	26	6	14-38	24	5	14-34
SV [ml]	108	18	72-144	87	15	57-117
SV/BSA [ml/m^2]**	54	6	42-66	52	7	38-66
EF [%]	67	5	57-77	67	5	57-77
Mass [g]	134	21	92-176	98	21	56-140
Mass/BSA [g/m^2]	67	9	49-85	61	10	41-81

Table 2 The weighted normal mean values for left ventricular parameters (ages 20-80) with CMR (41)

LV papillary muscle mass *included as part of LV mass*. Pooled weighted mean values from references (50-52). $Mean_p$ = pooled weighted mean; SD_p = pooled standard deviation; * = calculated as $mean_p \pm 2*SD_p$; EDV = end-diastolic volume; ESV = end-systolic volume; SV = stroke volume; EF = ejection fraction; BSA = body surface area; SD = standard deviation; **from references (51, 52) only.

The largest of these studies to date was the UK Biobank where 5065 healthy participants underwent CMR scanning using SSFP at 1.5 Tesla. This confirmed the changes in LVM with age

and gender (53). Given the variations in LVM with age and sex some groups including Maceira et al have produced age-sex specific ranges as shown in Table 3 (51).

	Men		Women	
Parameter	<60 years	≥60 years	<60 years	≥60 years
EDV [ml]	161 ± 21 (119, 203)	148 ± 21 (106, 190)	132 ± 21 (90, 174)	120 ± 21 (78, 162)
EDV /BSA [ml/m^2]	82 ± 9 (64, 100)	76 ± 9 (58, 94)	78 ± 8.7 (61, 95)	69 ± 8.7 (52, 86)
ESV [ml]	55 ± 11 (33, 77)	48 ± 11 (26, 70)	44 ± 9.5 (25, 63)	38 ± 9.5 (19, 57)
ESV/BSA [ml/m^2]	28 ± 5.5 (17, 39)	25 ± 5.5 (14, 36)	26 ± 4.7 (17, 35)	22 ± 4.7 (13, 31)
SV [ml]	106 ± 14 (78, 134)	100 ± 14 (72, 128)	88 ± 14 (60, 116)	82 ± 14 (54, 110)
SV/BSA [ml/m^2]	55 ± 6.1 (43, 67)	52 ± 6.1 (40, 64)	52 ± 6.2 (40, 64)	47.5 ± 6.2 (35, 60)
EF [%]	66 ± 4.5 (57, 75)	68 ± 4.5 (59, 77)	67 ± 4.6 (58, 76)	69 ± 4.6 (60, 78)
Mass [g]	147 ± 20 (107, 187)	145 ± 20 (105, 185)	106 ± 18 (70, 142)	110 ± 18 (74, 146)
Mass/BSA [g/m^2]	74 ± 8.5 (57, 91)	73 ± 8.5 (56, 90)	62 ± 7.5 (47, 77)	63 ± 7.5 (48, 78)

Table 3 Left ventricular parameters, by age and gender (51)

LV papillary muscle mass *included as part of LV mass* from reference

* = calculated as mean ±2*SD; EDV = end-diastolic volume; ESV = end-systolic volume; SV = stroke volume; EF = ejection fraction; BSA = body surface area; SD = standard deviation.

With the increased use of 3 Tesla MR systems there has been a need to establish equivalent data from images acquired at this higher field strength. Imaging at a higher field strength has a number of effects including changes in relaxation times and signal to noise ratio (SNR) and CNR. T1 relaxation times increase with field strength whilst changes in T2 relaxation times are negligible and a number of CV studies have significant T1 weighting (54, 55). Images acquired at 3 Tesla have shown increased SNR for myocardium and blood as well as increased blood to myocardium CNR (56, 57). Therefore, it is conceivable that myocardial boundary delineation could be perceived differently at 1.5 Tesla and 3.0 Tesla. To date, comparative analyses of LV indexes, mass and volume have shown no significant difference in the values obtained at 3.0 Tesla compared to 1.5T (58, 59). Gandy et al scanned a population of 1528 volunteers using SSFP sequences at 3 Tesla. They demonstrated that the LV reference ranges were similar to those described at 1.5 Tesla including the previously observed variations with age and gender. Table 4

shows the age-sex matched absolute data and Table 5 shows the normalized LV structure and function data acquired (60).

Absolute	No Volunteers	EF (%)	EDV (ml)	ESV (ml)	SV (ml)	LVM (g)
All	1515 (100%)	69 ± 6	133 ± 29	42 ± 15	91 ± 19	103 ± 29
Males	574 (37.9%)	68 ± 6	155 ± 28	50 ± 15	105 ± 19	129 ± 24
Females	941 (62.1%)	69 ± 7	120 ± 21	37 ± 12	82 ± 14	87 ± 17
Males (40s)	197 (13.0%)	67 ± 6	163 ± 27	54 ± 13	109 ± 20	135 ± 27
Males (50s)	235 (15.5%)	68 ± 6	153 ± 27	49 ± 15	104 ± 18	128 ± 22
Males (60s)	118 (7.7%)	68 ± 7	147 ± 26	47 ± 15	100 ± 17	123 ± 21
Males (≥70s)	24 (1.6%)	68 ± 6	143 ± 32	47 ± 15	97 ± 21	122 ± 24
Females (40s)	318 (21.0%)	68 ± 6	127 ± 20	41 ± 11	86 ± 14	88 ± 17
Females (50s)	371 (24.5%)	69 ± 7	121 ± 21	38 ± 12	83 ± 14	88 ± 17
Females (60s)	213 (14.1%)	71 ± 7	110 ± 19	33 ± 12	78 ± 12	84 ± 16
Females (≥70s)	39 (2.6%)	72 ± 6	104 ± 18	30 ± 10	74 ± 12	81 ± 15

Table 4 Age-sex matched absolute LV structure and function values acquired with 3 Tesla CMR (60)

The presented data (mean ± SD) are stratified by gender and also age decades. EF = ejection fraction, EDV = end diastolic volume, ESV = end systolic volume, SV = stroke volume, LVM = left ventricular mass, EDVI = end diastolic volume index, ESVI = end systolic volume index, SVI = stroke volume index, LVMI = left ventricular mass index.

Normalised	No Volunteers	EF (%)	EDVI (ml/m^2)	ESVI (ml/m^2)	SVI (ml/m^2)	LVMI (g/m^2)
All	1515 (100%)	69 ± 6	71 ± 13	22 ± 7	49 ± 8	55 ± 12
Males	574 (37.9%)	68 ± 6	77 ± 13	25 ± 7	52 ± 9	64 ± 10
Females	941 (62.1%)	69 ± 7	68 ± 11	21 ± 7	46 ± 7	49 ± 8
Males (40s)	197 (13.0%)	67 ± 6	80 ± 13	26 ± 7	53 ± 9	66 ± 12
Males (50s)	235 (15.5%)	68 ± 6	76 ± 13	24 ± 8	52 ± 9	63 ± 10
Males (60s)	118 (7.7%)	68 ± 7	74 ± 13	24 ± 8	51 ± 9	62 ± 10
Males (≥70s)	24 (1.6%)	68 ± 6	73 ± 14	24 ± 7	49 ± 9	62 ± 10
Females (40s)	318 (21.0%)	68 ± 6	70 ± 10	22 ± 6	48 ± 7	49 ± 8
Females (50s)	371 (24.5%)	69 ± 7	68 ± 11	21 ± 7	47 ± 7	49 ± 8
Females (60s)	213 (14.1%)	71 ± 7	64 ± 10	19 ± 7	45 ± 7	49 ± 8
Females (≥70s)	39 (2.6%)	72 ± 6	61 ± 9	18 ± 5	43 ± 6	48 ± 8

Table 5 Age-sex matched normalised LV structure and function values acquired with 3 Tesla CMR (60)

The presented data (mean ± SD) are stratified by gender and also by age decades. Normalization of the absolute values to body surface area was performed using the Mosteller formula.

1.2.5 Prevalence of Left Ventricular Hypertrophy

The Framingham heart study offered an opportunity to assess the prevalence of LVH in the general population (61). LVH was found in 16% of men and 19% of women of their cohort. The prevalence increased significantly with age occurring in 33% of men and 49% of women over 70. There was a significant association between blood pressure (BP) and LVH even at levels of systolic BP below 140mmHg (age adjusted). Across the range of observed body mass index (BMI) values, there was a tenfold increase in prevalence of age adjusted LVH in men, and a ninefold increase in women. Multivariate analysis showed age, BP, obesity, valve disease and MI to all be independently associated with LVH in both sexes. Clearly LVH is common for which several risk factors in addition to hypertension are implicated. Indeed, BP only contributes 25% to the variability of LV mass seen in a population (62).

In addition to the risk factors described above diabetes has been identified as another independent predictor of LVH. In the Framingham population, LVM was on average 22% higher in women with diabetes than in those without diabetes (16). Devereux et al compared the LV measurements between 1810 participants with diabetes and 944 without diabetes. Measures of LV mass indexed for BSA or height$^{2.7}$ were greater by 6% to 9% and by 12% to 14% respectively, in diabetic compared with non-diabetic women and men (18).

A local survey performed on 173 patients attending diabetic clinics in Ninewells found LVH to be present in 57 cases (32%). This was independent of BP or the use of angiotensin converting enzyme ACEi (63).

Dawson et al performed echocardiography on 500 patients with type 2 diabetes to identify LV abnormalities (17). At the time of publication of their results measurements were applied to the old ASE guidelines for defining Echo LVH. These defined LVH as a LVMI greater than 110g/m^2 in women and greater than 134g/m^2 in men and LVMI to height as greater than 47g/m$^{2.7}$ in women and greater than 50g/m$^{2.7}$ in men. Despite these higher cut offs LVH was found to be highly prevalent. LVM was successfully measured in 371 patients with LVH being identified in 264 (71%) when LVMI to height$^{2.7}$ and 159 (43%) when LVMI to BSA.

The increased prevalence of LVH in patients with diabetes in comparison to those without the disease is also demonstrated in studies using ECG criteria. Barrios et al aimed to assess the prevalence of LVH detected by different ECG criteria (64). At baseline, 37.5% of diabetic and

26.4% of non-diabetic patients fulfilled criteria of ECG-LVH by Cornell product (Cornell voltage x QRS duration) (p=0.02), 25.7% and 23.2%, respectively, by Sokolow-Lyon product (Sokolow-Lyon voltage x QRS duration) (p=0.18), 11.8% and 13.7% by Cornell voltage (p=0.16), and 14.3% and 11.6% by Sokolow-Lyon voltage (p=0.10). The lower overall estimates of LVH prevalence compared to similar studies using echocardiography is understandable as the classic Sokolow-Lyon and Cornell Voltage criteria are known to underestimate LVH compared to Echo and therefore are less sensitive as discussed in the previous chapter. (21, 22)

1.2.6 Increased Cardiovascular Risk Associated with Left Ventricular Hypertrophy

It has consistently been shown that LVH detected on ECG and Echo is strongly associated with increased risk for multiple manifestations of CVD (65-67). Early reports from Framingham described a strong association between Echo determined LVM and the incidence of coronary heart disease in elderly people (68). Levy et al found that the adverse prognostic implications of increased LVM also applied to a younger population (27). They followed up 3,220 patients enrolled in the Framingham Heart Study over four years, all of whom were over 40 years old and had no history of CVD. The relative risk (RR) of CVD was 1.49 in men for each 50g/m^2 increment in LVM (95%CI, 1.20 to 1.85) whilst in women it was 1.57 (95% CI, 1.20 to 2.04). LVM also conferred an increased risk for death from all causes with a RR of 1.49 (95%CI, 1.14 to1.94) in men and 2.01 (95% CI, 1.44 to 2.81) in women (27).

Haider et al performed longer follow up of the same cohort to specifically assess the relationship of Echo determined LVH to the risk of sudden death. The adjusted hazard ratio (HR) for sudden death was 1.45 (95%CI 1.10 to 1.92, p=0.008) (69).

Hypertension is a major risk factor for both LVH and CVD. This has led to the belief that the risk associated with LVH simply reflects the risk associated with hypertension. The adverse prognosis of LVH however, is evident irrespective of the presence of hypertension. Brown et al followed up nearly 8,000 patients over 17 years from the second National Health and Nutrition Examination Survey, (NHANES II). They confirmed the findings that the presence of LVH is a strong predictor of future CVD. Significantly though normotensives with LVH had survival similar to hypertensive

adults with LVH. They also had lower survival than normotensive and hypertensive adults with no LVH (70).

LVH is therefore a clear independent predictor of CV death yet is currently ignored compared to CAD and left ventricular systolic dysfunction (LVSD). This is despite a head to head study by Liao et al which directly compared the survival of Echo determined LVH with CAD and LVSD. The study included 1089 black patients who underwent both coronary angiography and Echo. Multivariate analysis showed LVH to be the biggest risk factor for death with a RR of 2.4 compared to 2.0 and 1.6 for LVSD and multivessel coronary disease respectively (71).

Whether there is a sex and gender differential in the impact of LVH on mortality is a matter of some debate. The RR associated with LVH is higher in blacks than whites and in women than men (23, 27, 71, 72). LVH is however also more prevalent in blacks than whites and in women than men and is likely to account for much of the race and gender differences in prognosis (73).

Vakili et al performed a large meta-analysis to provide a comprehensive review of the relationship between LVH and future adverse clinical outcomes (74). Overall, 20 studies between 1960 and 2000 encompassing 48,545 patients diagnosed with LVH on ECG, Echo or both were included in the analysis. The ECG LVH studies evaluated 14,450 subjects and found that LVH was associated with an adjusted RR of CV events of 1.6 to 4.0. The Echo LVH studies evaluated 3,651 subjects and also showed a strong relation between LVH and future CV events with LVH associated with a RR of 1.5-3.5. The overall weighted adjusted RR of future CV events on both the ECG and Echo studies combined was 2.3. It should be noted that in each study the definition of CV events differed as did the ECG and Echo criteria for defining LVH. Nevertheless, the meta-analysis shows a strong relationship between LVH and adverse CV events. This is summarised in Figure 1 (74).

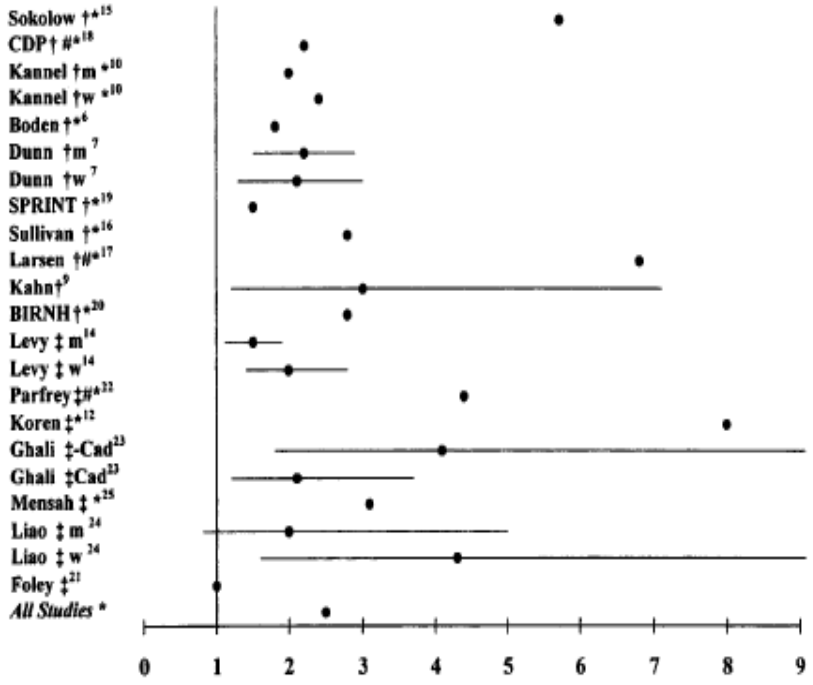


Figure 1 Summary of the mean adjusted risk of baseline LVH for all-cause mortality meta-analysis performed by Vakili et al (74)

Mean relative risk is represented by a solid circle and when available, 95% confidence interval are represented by horizontal lines. Cad coronary artery disease; -Cad without coronary disease; †Electrocardiographic LVH; ‡ Echocardiographic LVH; # unadjusted; m=in men; w= in women; *P<0.05.

Assuming a population prevalence of LVH of around 20% and an adjusted RR of around 2.0 it can be assumed that about one sixth of CV events are associated with LVH (69).

The mechanisms by which cardiac hypertrophy may promote this increased CV morbidity and mortality are discussed below.

1.2.7 Mechanisms of the Increased Cardiovascular Risk with Left Ventricular Hypertrophy

1.2.7.1 Myocardial Ischaemia and Left Ventricular Hypertrophy

Of the reasons for the increased CV risk associated with LVH, ischaemia is the one that has been appreciated the longest. Even without co-existing epicardial atherosclerotic coronary arterial disease ischaemia related to hypertensive heart disease has been a classical cause of angina pectoris (75). Andren et al performed exercise tests on elderly men with no known CAD with either LVH and hypertension, or LVH and a normal BP and compared these with healthy controls (76). Overall 58 patients were included in the study. More pronounced ST depression occurred on exercise testing in the hypertensive subjects when compared to the healthy group without LVH. In both of the LVH groups, more than 20% of the subjects had ST-segment depression greater than or equal to one millimetre, compared with only 5% of the healthy group without LVH.

In patients with LVH ischaemia can occur despite no obstructive epicardial CAD for many reasons:

At a macrovascular level, when LVH develops the LV end diastolic pressure increases which increases the transmural pressure during diastole and as almost all cardiac blood flow occurs in this phase of the cardiac cycle this compromises coronary blood flow which in turn causes ischaemia (77). Hypertension is the commonest cause of LVH and increased arterial stiffness is often seen in long standing hypertension which accelerates aortic pulse wave velocity (78). This results in an earlier return of reflected waves from the periphery of the aorta, increasing LV afterload and central pulse pressure. This occurs with concomitant fall in central diastolic pressure decreasing coronary perfusion therefore contributing to myocardial ischaemia.

In addition it is thought that patients with LVH also have coronary microvascular dysfunction (CMD) and impaired coronary flow reserve (79). There is no technique to directly visualise the coronary microcirculation but many experimental and clinical studies have demonstrated impaired coronary blood flow and flow reserve in patients with hypertension despite normal coronary angiograms (80-83). More recently sophisticated techniques have emerged using pharmacological

interventions to allow precise quantification of coronary flow reserve. These interventions include vasodilators such as adenosine and dipyridamole. Some have been used alongside radioisotopic techniques to quantify myocardial perfusion in addition to coronary flow. Fu et al aimed to assess coronary flow reserve in 47 hypertensive patients with normal coronary angiograms. The hypertensive patients were divided into two groups defined by the presence or absence of LVH (84). 17 normal cases were also included as controls. All patients underwent an adenosine ^{99M}Tc-MIBI single-photon computed tomographic (SPECT) myocardial perfusion scan. There was a statistically significant decrease in coronary reserve in the hypertensive group following adenosine infusion and this was especially remarkable in the patients with LVH. In the LVH group 17 cases (77.3%) developed myocardial ischaemia in comparison to 9 cases (36%) in the control group.

There are many proposed reasons for this coronary microcirculation dysfunction. At the arteriolar level in hypertrophied hearts there is marked thickening of the intramural coronary arterioles due to intimal hyperplasia and medial hypertrophy significantly reducing the luminal area (85). The LVH itself is implicated as it's associated with expansion of the extravascular space due to increased extravascular interstitial fibrosis which can cause mechanical compression of the coronary vasculature (84). Other factors include endothelial dysfunction secondary to the vascular remodelling described above which limits the ability of the coronary arteries to dilate. Endothelial nitric oxide (NO) is a potent physiological vasodilator and there is evidence that NO-mediated endothelial dysfunction occurs in hypertension (77, 86-88). Such dysfunction in endothelium mediated vasodilatation plays an important part in the functional abnormalities of resistance vessels that are seen in hypertensive patients and contributes to LVH and ischaemia (77, 89).

This diminished coronary flow reserve renders the myocardium more vulnerable to episodes of ischaemia during times of increased cardiac work or reductions in perfusion pressure. With the development of cardiac magnetic resonance imaging (CMRI) pathophysiological information regarding the long-term consequences of ischaemia has been acquired albeit predominately in patients with hypertrophic cardiomyopathy (HCM). Late gadolinium studies allow non-invasive tissue discrimination by identification of interstitial and replacement fibrosis (90). CMR studies in large HCM cohorts have shown that as many as 80% of them demonstrate areas of late gadolinium enhancement in variable patterns suggestive of localised fibrosis occupying on average 10% of the

overall LV myocardium (91, 92). Significant CMD has been demonstrated in the LV segments demonstrating this late gadolinium enhancement (93). This suggests that CMD in LVH may over time lead to recurrent episodes of ischaemia resulting in myocyte injury eventually leading to replacement fibrosis (94). Such fibrosis and scarring can contribute to diastolic heart failure (impaired relaxation), systolic heart failure (impaired contractility), as well as act as a substrate for arrhythmias. All of these are well recognised adverse effects of LVH and contribute to the poor prognosis in patients with LVH. The proposed pathophysiological events linking CMD in patients with LVH to long term adverse clinical outcome is summarised in Figure 2 (85).

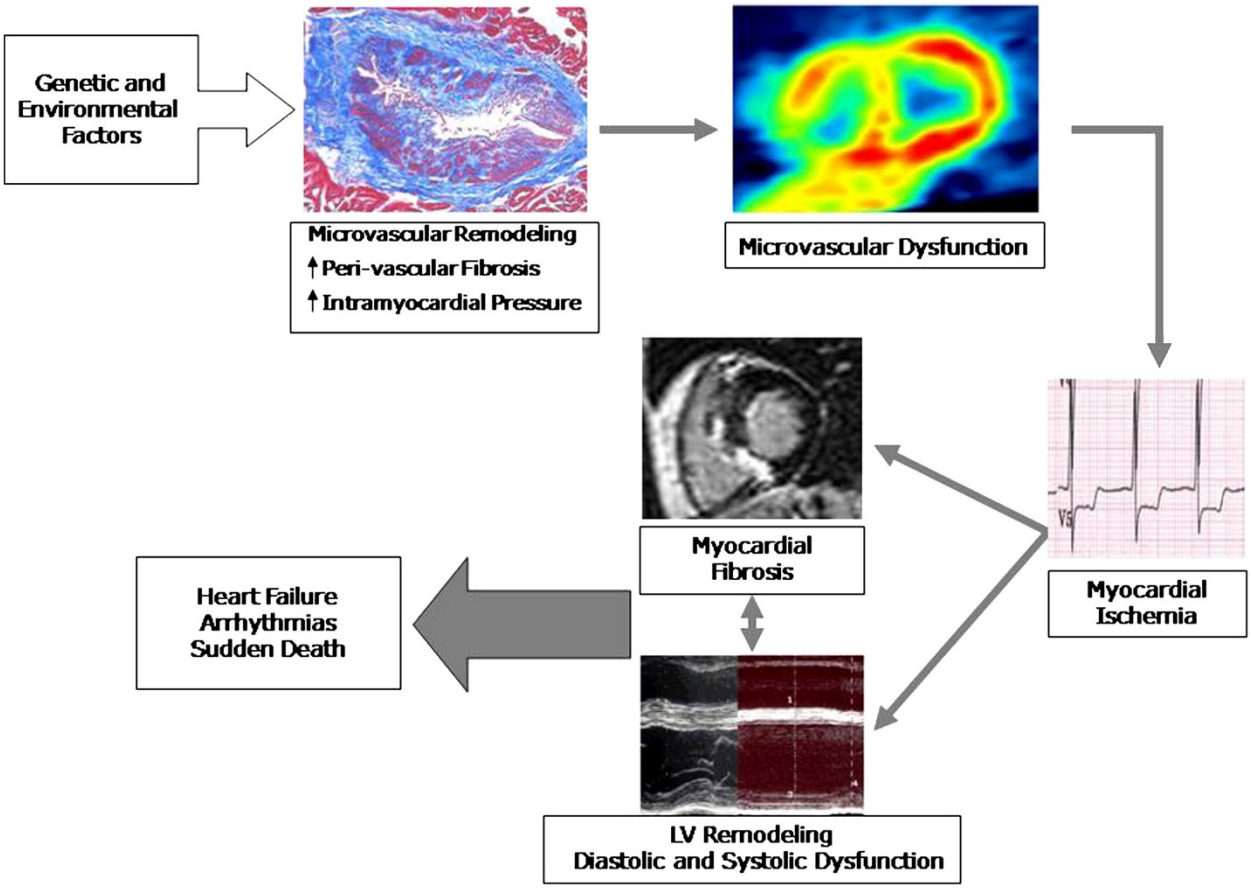


Figure 2 The pathophysiological events linking coronary microvascular dysfunction in patients with LVH to the long term adverse clinical outcomes (85)

With regards coronary flow reserve in patients with diabetes, there is consistent evidence that coronary flow reserve is impaired in diabetes (95-97). This vascular dysfunction is present even in the absence of other diabetes associated coronary risk factors such as hypertension and

dyslipidaemia (97, 98). Di Carli et al investigated the coronary vascular function in 35 young subjects with diabetes with no overt CV complications and compared it to 11 age matched healthy controls (99). They demonstrated marked CMD in response to both adenosine infusion and to a cold pressor test in comparison to the healthy controls. Mizuno et al also demonstrated that diabetic patients had exercise induced delayed onset of LV relaxation in association with CMD in the absence of known co-existent heart disease (100).

In summary there is evidence that CMD occurs in diabetic patients and when combined with LVH and hypertension there is a significant impact on coronary artery flow and hence ischaemia.

1.2.7.2 Arrhythmias and Left Ventricular Hypertrophy

As discussed above LVH is a powerful predictor not only for total and CV mortality but also sudden cardiac death (69). An important reason for this is the association of LVH with ventricular arrhythmia (101). Patients diagnosed with either ECG or echocardiographic LVH are more likely to have ventricular ectopics or ventricular arrhythmias compared with normotensive or hypertensives without LVH (102, 103). In a meta-analysis of 10 studies involving over 27,000 patients, the occurrence of ventricular tachycardia or fibrillation was significantly greater when LVH was present (odds ratio 2.8 compared with no LVH: 95% CI 1.8-4.5) (104). Irrespective of how LVH is diagnosed, LVH is associated with an increased risk for sudden cardiac arrest (105)
The relationship of ventricular arrhythmias with LVH was studied in over 6,000 Framingham heart study patients (106). Echocardiographic LVH was associated with an increased risk of each of the six ventricular arrhythmia grades (Lown grading system for ventricular arrhythmias). After adjustment for, age, sex, systolic BP, valvular heart disease, angina, acute MI this association remained significant.
Ghali et al were the first to show that the association of LVH with ventricular arrhythmias occurred in patients with normal coronary arteries (76). The occurrence of ventricular arrhythmia was investigated using 24 hour ambulatory ECG monitors in 49 hypertensive patients with LVH on echocardiography and normal findings on a diagnostic coronary angiogram. The frequency and complexity of ventricular arrhythmias was significantly related to the presence of LVH. In addition for every 1mm increase in either the inter ventricular septum or LV posterior wall there was a two

to three fold increase in the occurrence and complexity of ventricular arrhythmia. Schmieder et al also demonstrated that the frequency and severity of ventricular ectopy was related to the severity of LVH and chamber volume and LV contractility (107). Several studies have suggested that complex ventricular ectopy is predictive of all-cause mortality (108, 109). Even more importantly increases in QRS duration and QT_C prolongation are independently associated with sudden cardiac death and are ECG abnormalities commonly found subjects with LVH (110).
Electrophysiological testing has also confirmed the link between LVH and ventricular arrhythmia (111).

The reasons why LVH is proarrhythmic are multifactorial in origin. Fibrosis is one of the myocardial abnormalities associated with LVH and is likely the main substrate for the development of ventricular arrhythmia particularly re-entrant arrhythmia (112). Other proposed mechanisms associated with LVH include lengthening of the action potential duration, slower membrane repolarization and the generation of early and delayed afterdepolarizations which further increase the potential for ventricular arrhythmias (113-115).
As discussed above LVH is commonly associated with myocardial ischaemia even in the absence of CAD. Numerous studies have demonstrated that LVH exacerbates ischaemia induced arrhythmias suggesting that the combination of electrophysiological changes induced by LVH and myocardial ischaemia are important in creating the pro arrhythmic milieu (116-119).

1.2.7.3 Atrial Fibrillation

Left atrial enlargement which is common in people with LVH has been shown to be an important mechanism in the development of atrial fibrillation. When the left atrium dilates in response to external stressors such as hypertension, obesity and LVH, activation of fibroblasts with enhanced connective tissue deposition and fibrosis occurs in the process (120-122). This structural remodelling is thought to result in dissociation between muscle bundles and local conduction resulting in electrical instability favouring re-entry and perpetuation of arrhythmia (123, 124).

This predisposition towards the development of atrial fibrillation is important as AF is independently associated with a two-fold increased mortality in women and a 1.5 fold increase in men (125). AF is also associated with increased morbidity, such as stroke and heart failure (126, 127). Decreased quality of life and depressed mood are common in AF patients and between 10-40% are hospitalized each year (128-130).

Diabetes and atrial fibrillation frequently co-exist because of the association with other risk factors (131). However, it is becoming increasingly recognised that diabetes may also be an independent risk factor for atrial fibrillation. Observational studies have reported on the association between diabetes mellitus and atrial fibrillation with equivocal results (132-136). The largest of which as was a case control study carried out by Movahed et al who analysed 845,748 inpatient records from all the Veterans Health Administrations Hospitals in America (136). Atrial fibrillation occurred in 43,674 (14.9%) of the patients with diabetes mellitus vs 57,077 (10.3%) of the control group ($p<0.0001$). Atrial flutter occurred in 11,852 (4%) of the patients with diabetes mellitus vs 13,544 (2.5%) of the control group ($p<0.0001$). Even after multi-variant analysis to correct for differences in co-morbid conditions including chronic heart failure, CAD and LVH diabetes remained a strong independent risk factor for atrial fibrillation with an odds ratio of 2.13 (95% CI:2.10 to 3.16; $p<0.001$) and for atrial flutter with an odds ratio of 2.20, CI:2.15 to 2.26; $p<0.0001$). This study was in contrast to a large study carried out by Wilhelmsen et al who did not find a correlation between AF and diabetes (135).

Huxley et al performed a meta-analysis of published studies to further examine the association of diabetes mellitus with atrial fibrillation (137). Seven cohort and four case control studies with 108,703 cases of atrial fibrillation among 1,686,097 participants contributed to the analysis. They concluded that participants with diabetes had an approximate 40% greater risk of AF compared with non-diabetics (RR 1.39 (95% CI: 1.10-1.75) $p<0.001$). Studies that had adjusted for multiple risk factors reported a smaller effect compared with age adjusted studies, (RR 1.24 (95% CI: 1.06-1.44 vs 1.70 (1.29-2.22) $p =0.053$). Figure 3 summarises the findings of the meta –analysis.

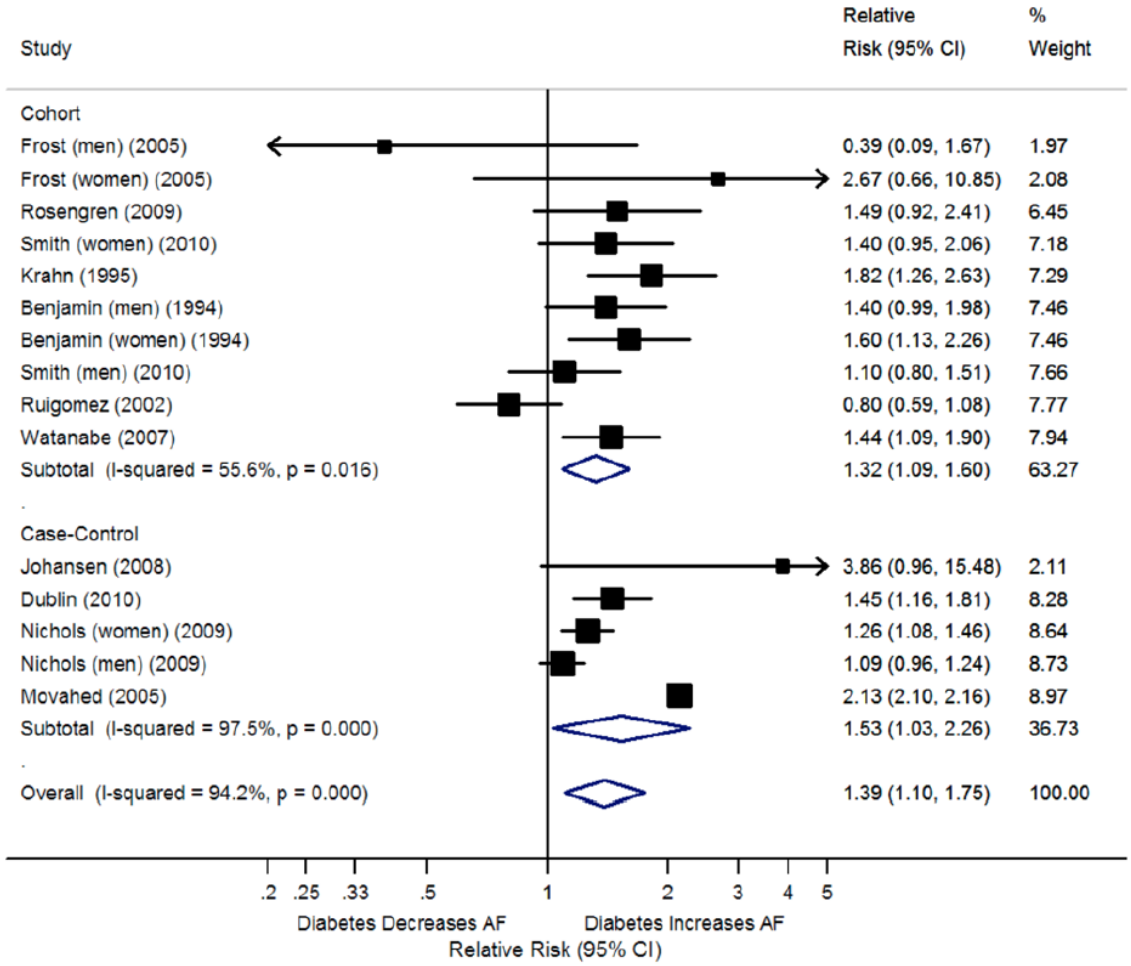


Figure 3 Summary of the relationship between AF and diabetes in the meta-analysis performed by Huxley et al(137)

The centre of each black square is the point estimate. The area of the square is proportional to the statistical size and each horizontal line shows the 95% confidence interval. The open diamonds represent the summary estimates from the pooled cohort and pooled case control studies

The predisposition for patients with diabetes to develop atrial fibrillation is also important as diabetes is a risk factor for stroke and other complications in atrial fibrillation (138).

A longer duration of diabetes in patients with atrial fibrillation also confers an increased risk of thrombo-embolism (139). Intensive glycaemic control does not appear to reduce the rate of new onset atrial fibrillation although metformin therapy may decrease the long term risk of atrial fibrillation and may reduce the stroke risk (138, 140, 141)

1.2.7.4 Left atrial dilatation and Left Ventricular Hypertrophy

Both eccentric and concentric LVH impair ventricular filling due to reduced compliance of the LV resulting in diastolic dysfunction. The left atrium therefore has to produce higher filling pressures to fill the ventricle which results in atrial remodelling and ultimately left atrial enlargement (142). Left atrial dilatation is a sensitive indicator of LV preload (143). Chronic pressure overload from hypertension or chronic volume overload related to obesity can therefore both result in left atrial enlargement (144). Therefore, it is not surprising that left atrial enlargement is strongly associated with LVH (145).

In addition to being an important mechanism in the development of atrial fibrillation, left atrial enlargement is important because it has also been found to independently predict the development of heart failure and other CV events (146-148).

Kizer et al investigated the prognostic value of left atrial diameter for predicting CV events by performing echocardiography on 2804 American Indians who were free of CVD, valvular disease and atrial fibrillation (148). Over a 7 year follow up 368 events occurred which included non-fatal stroke, coronary heart disease, chronic heart failure and death from CV causes. After multivariate analysis adjusting for other common CV risk factors such as age, BMI, hypertension, diabetes, high cholesterol, smoking and LVH, left atrial diameter remained an independent predictor of first CV events. An Echo sub study of the LIFE study showed similar findings in 881 hypertensive patients with ECG LVH during a 4.8 year follow up (149). Baseline left atrial diameter predicted CV events (defined as combined CV death, MI and stroke) with a HR of 1.98 (95% CI: 1.02-3.83) even when adjusted for significant effects of Framingham risk score and a history of atrial fibrillation. Other studies have demonstrated an independent relationship between LA diameter and chronic heart failure and left atrial size has been shown to be associated with sudden cardiac death in patients with heart failure (146, 150).

Large population based studies including the Framingham Heart Study have suggested that left atrial enlargement is associated with incident stroke irrespective of diagnosed atrial fibrillation (150).

All participants in the Framingham Heart Study over 50 years old were followed up over 8 years and following multivariate adjustment it was shown that for every 10-millimetre increase in left atrial size, the RR for stroke was 2.4 in men and 1.4 in women.

1.2.7.5 Heart Failure

As will be discussed in more detail below LVH involves changes in the myocardial tissue architecture consisting of perivascular and myocardial fibrosis in addition to myocyte hypertrophy (151). The LV fibrosis reduces the LV distensibility, filling and relaxation. Whilst diastolic dysfunction can occur in the absence of LVH, the degree of diastolic dysfunction is usually correlated with LVM (152). Diastole consists of two phases an early rapid filling phase secondary to active myocyte relaxation and a late passive phase which is dependent upon the elastic properties of the left ventricle. It has been shown that the velocity of relaxation of hypertrophied myocytes is reduced and contributes to diastolic dysfunction (152). This is an active process and therefore energy requiring meaning it is impaired in the presence of ischaemia, which as discussed above is common in subjects with LVH. Passive relaxation is determined by the degree of myocardial fibrosis. Brilla et al showed that when myocardial fibrosis was eliminated with treatment with an ACE inhibitor normalisation of diastolic stiffness occurred (153). Sugihara et al demonstrated an inverse correlation between rapid LV filling volume and the percentage of myocardial fibrosis as assessed by right ventricular endomyocardial biopsy (154)

When impairment of diastolic dysfunction occurs, LV end-diastolic pressure increases which results in elevated pressures within the pulmonary capillaries (unless there is severe mitral stenosis). This can produce dyspnoea and other symptoms and signs of chronic heart failure even in the presence of normal left ventricular ejection fraction (LVEF) (155).

It has long been assumed that LVH with a normal ejection fraction (EF) is a precursor to LVSD and dilated cardiomyopathy (156). The classical teaching of the cardiac structural and functional changes in hypertensive heart disease is that hypertension leads to concentric LVH followed by dilated cardiomyopathy commonly referred to as the “burnt out ventricle”. Meerson was the first to describe this transition following his work with animal models in the 1960s (157). Several lines of evidence have supported this theory. First, the transition from concentric LVH to systolic

dysfunction is observed in animal models including spontaneously hypertensive rats and rats with LVH due to banding of the ascending aorta (158, 159). Second, hypertension is a major risk factor for the development of LVSD particularly in African Americans (160). Progression from LVH to dilated cardiomyopathy has been demonstrated in patients with aortic stenosis and HCM (161, 162). Finally with the development of more sophisticated measures of LV function, patients with hypertension commonly have impaired LV mid wall shortening and peak systolic mid wall circumferential strain despite a normal EF. This suggests the process of dilated cardiomyopathy has begun (163-165).

Despite this it remains unclear if this conversion is part of the natural history of hypertension-induced LVH in humans. It is not known how well the natural history in animal models or patients with aortic stenosis or HCM replicates that of hypertensive heart disease. The close association between hypertension, LVH, CAD and heart failure complicates the question. Several studies have tried to answer how frequently patients with concentric LVH develop systolic heart failure in the absence of CAD or interval MI. Drazner et al assessed 159 middle aged hypertensive patients who had LVH with normal LV systolic function and a follow up Echo performed for clinical indications at least one year after the baseline Echo(166). Over an average follow up period of around 4 years only 18% (28) developed left LVSD. In addition, nearly half of these had an interval MI.

The Cardiovascular Health Study 3402 participants with LVH and a normal LVEF had a repeat Echo after a five year follow up period (167). Increased LV mass was found to be a risk factor for the transition to LVSD independent of CAD and MI. However, it was eccentric not concentric LVH that was associated with this outcome.

In summary, evidence is still lacking that LVH resulting from hypertension is a definite major risk factor for systolic heart failure in the absence of CAD. However, LVH is a definite precursor for heart failure in the form of diastolic dysfunction.

1.2.7.6 Summary

The development of LVH in a patient with diabetes is a worrying sign. LVH has been consistently shown to be associated with an increased risk of many manifestations of CVD. This is on top of the already heightened CV risk associated with diabetes. LVH affects coronary flow inducing

ischaemia. LVH increases the risk of arrhythmias such as ventricular tachycardia and atrial fibrillation both of which are associated with significant morbidity and mortality. LVH impedes ventricular filling which can result in left atrial dilatation which is a CV risk factor in its own right. Finally, the impaired ventricular filling with LVH leads to diastolic heart failure which may develop into systolic heart failure over time.
As a consequence, LVH in diabetic patients should be seen as an important target for future therapies.

1.3 The Development of Left Ventricular Hypertrophy

1.3.1 Introduction

The myocardium has three morphological compartments, the muscular compartment consisting of myocytes, the interstitial component formed by fibroblasts and collagen and the vascular compartment with smooth muscle and endothelial cells (168). As touched upon above LVH occurs in response to both haemodynamic and biomedical stress from either intrinsic or extrinsic sources. It is generally believed that these stressors initiate a cascade of biological events leading to cardiac hypertrophy(20). The increased mass is due to cardiomyocyte hypertrophy, rather than hyperplasia. This is because cardiomyocytes are terminally differentiated shortly after birth (20). Cardiomyocyte hypertrophy is but one of many alterations in LVH. Fibroblasts also undergo hyperplasia and conversion to myofibroblasts leading to an increased deposition of interstitial and perivascular collagen (169). Hypertrophy of vascular smooth muscle cells also occurs and arteriolar thickening is also characteristic of the hypertrophied heart (86)

Hypertension is strongly correlated with systolic hypertension due to the increased afterload on the heart (170). However, studies have also shown a number of other variables to be independently associated with LVH including obesity, age and diabetes. BP lowering trials have shown different antihypertensives regress LVH more than others independent of BP. Agents which block renin angiotensin aldosterone system (RAAS) such as ACE inhibitors (ACEi) and Angiotensin Receptor blockers (ARBs) have been shown to regress LVH more than other agents such as beta-blockers (171-173). There is also only a modest correlation between BP and LV mass suggesting additional important factors for the development of LVH.

It is now understood that LVH is not only mediated by the mechanical stress of pressure overload but also by various neurohormonal substances that independently exert trophic effects on myocytes in the heart (155). These trophic factors include angiotensin II, aldosterone, noradrenaline, leptin as well as insulin (170). These diverse stimuli induce hypertrophy by a common pathway by activating a variety of mitogen activated protein kinases (MAPK) such as extracellular signal-regulated protein kinase 1 and 2 (ERK1/2) and p38 (174). This results in a production of a series of cytokines and growth factors that stimulate protein synthesis and re-expression of embryonic cardiac genes such as β myosin heavy chain and a subsequent increased in cardiac myocyte size. The hypertrophic response to these hypertrophic stimuli appears to be also mediated by reactive oxidative species (ROS) (175). The extent of cardiac growth and response to increased pressure loading is not consistent among patients suggesting genetic mechanisms also contribute to cardiac hypertrophy (176).
The interplay of all these factors and the pathogenic theories for the development of LVH are discussed in turn below.

1.3.2 Blood Pressure

An increase in LV wall stress is one of the principal factors in the development of LVH (168). This can occur secondary to volume overload (e.g. mitral and aortic regurgitation) although most commonly it is caused by pressure overload caused by the heart pumping against an increased afterload due to hypertension. In the short term increasing LVM is beneficial as increasing LV wall thickness in proportion to increased pressure helps to normalize myocardial stress (177). Therefore, hypertension is a powerful determinant of LV mass (61, 178, 179) .

Although hypertension is the leading cause of LVH, there is poor correlation between the severity of the hypertrophy and the measured BP. The haemodynamic load on the left ventricle cannot be assessed with a single BP measurement.
Therefore it is not surprising that ambulatory blood pressure monitoring (ABPM) is superior at predicting end organ damage associated with hypertension when compared to clinic BP measurements (180). Far better correlations have been demonstrated between LVM and 24 hour ABPM (181, 182). Verdecchia et al performed ABPM and echocardiography in 165 untreated

hypertensive patients and 92 healthy subjects (183). In the hypertensive group, LVMI showed a closer correlation with the ABPMs than the office measurements. Hypertensive patients were also classified according to the difference between their observed and predicted levels of ambulatory BP. When compared to those with lower than predicted ambulatory BP patients those with higher than predicted ambulatory BP had higher values of LVMI with a prevalence of LVH of 6-10% in the former and 35-39% in the latter.

The principal advantage of ABPM is the number of readings obtained in the outpatient setting. Frequent readings not only allow a closer estimate of "true BP" it allows for the analysis of blood pressure variability (BPV). BP classically varies from time to time during the day and between days, months and seasons and this is known as BPV.

In addition to the duration and severity of hypertension the clinical manifestations of hypertension are also determined by diurnal variations of BP and overall 24 hour variability (184-186). The absence of the usual nocturnal fall in BP is associated with increased LVM (187). There is also an expanding body of evidence that BP variability is of prognostic importance independent of absolute mean BP levels (188). The extent of BP variability is independently associated with the rates of CV events and the extent of target organ damage (including LVH). In the PAMELA study 1648 participants underwent ABPM and echocardiography. BPV was obtained by calculating the standard deviation of the 24 hour mean and there was a positive relationship between LVMI and BPV which persisted after adjustment for gender, age and 24 hour average BP values (189).

1.3.3 Obesity

There is an abundance of evidence that obesity is associated with structural and functional changes in the heart including LVH (190). Adults are generally considered obese if they have a BMI of 30 or above.

The association between obesity and LVH could occur because obese patients have a high prevalence of hypertension with around 60% of obese subjects having hypertension (191). The combination of hypertension and obesity is additive with a 17-fold increased risk of developing

LVH (61, 179, 192). However, obesity has been shown to be independently associated with LVH (193-195).

Obesity can lead to the development of LVH by direct actions on the myocardium or indirectly by influencing a number haemodynamic and metabolic factors (196). This is summarised by Abel et al in Figure 4.

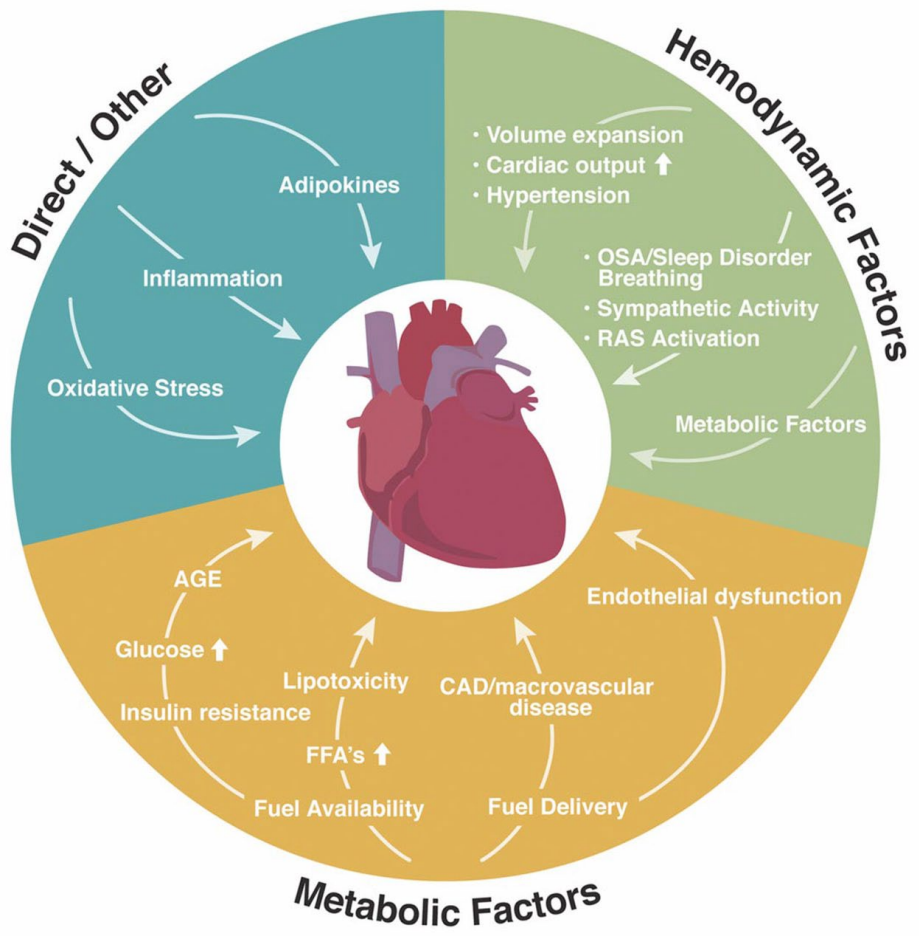


Figure 4 The role of obesity in the development of LVH(196)

Obese individuals have expanded central blood volume (197) . The increase in both lean and fat mass increases the metabolic demand resulting in a hyperdynamic circulation with increased blood volume (198). In addition to this increased preload LV afterload is increased in obese subjects due to increased peripheral resistance and greater conduit stiffness (199). These altered cardiac loading conditions can directly lead to an increase LVM.

Adipose tissue was once considered a simple lipid storage depot however, it is now known to produce a range of cytokines known as adipokines (200). Leptin in the most abundant adipokine produced by adipocytes (201). In humans, leptin concentration are correlated with adiposity (202). This is partly due to the increased adipose tissue to secrete leptin and due to selective leptin resistance (203). Obese people are resistant to leptin's central satiety actions but not to the effects on the sympathetic nervous system (SNS) (204). In lean individuals leptin circulates in the blood at a concentration of 5 to 15ng/ml whilst the concentration may reach up to 50 ng/ml in obese subjects (205). Several studies have revealed it has numerous effects on the CV system (206, 207). Indeed the functional leptin receptor Ob-Rb is found in the myocardium allowing it exert its action on the heart (208).

The role of leptin in the development of LVH is controversial with some researchers believing leptin promotes LVH whilst others believe it attenuates LVH. Leptin may directly lead to cardiac myocyte hypertrophy and studies have shown that leptin upregulates the MAPK cascades implicated in LVH (209). Leptin also significantly increases the expression of myosin light chain 2 and α-skeletal actin both known to be upregulated in LVH. Importantly this occurred at concentrations well within those found in obese subjects. Leptin has also been shown to functionally activate hemopoietic and embryonic cells promoting myocyte growth (210). In contrast, studies of leptin deficient mice have showed reversal of myocyte hypertrophy during leptin administration (211).

Leptin though may also exert its effects on the heart indirectly. Leptin is known to increase the secretion of aldosterone by the adrenal glands and augment the effect of angiotensin II (212-214). Leptin also stimulates the SNS particularly renal sympathetic nerves (203). This has numerous effects, first of which is the activation of MAPK cascades causing cardiac hypertrophy and fibrosis (174). Overexpression of the renin angiotensin-aldosterone system (RAAS) and SNS increases peripheral resistance and promotes sodium and water retention. Sodium and water retention is further exacerbated by the decreased activity of natriuretic peptides due to the up regulation of neprilysin by renal sympathetic nerve activation (215). Leptin clearly contributes to the plasma volume expansion observed in obese individuals. Indeed, this and the increased SNS stimulation is why leptin is associated with hypertension. Chronic leptin administration has been shown to increase BP and fasting plasma leptin concentrations have been found to be significantly higher in

hypertensive subjects than in normotensive controls (216-218). It's important to point out though that plasma leptin concentrations have been associated with increased LVM independent of BP levels (218).

A summary of the possible mechanisms by which leptin contributes to adverse cardiac remodelling is shown in the Figure 5.

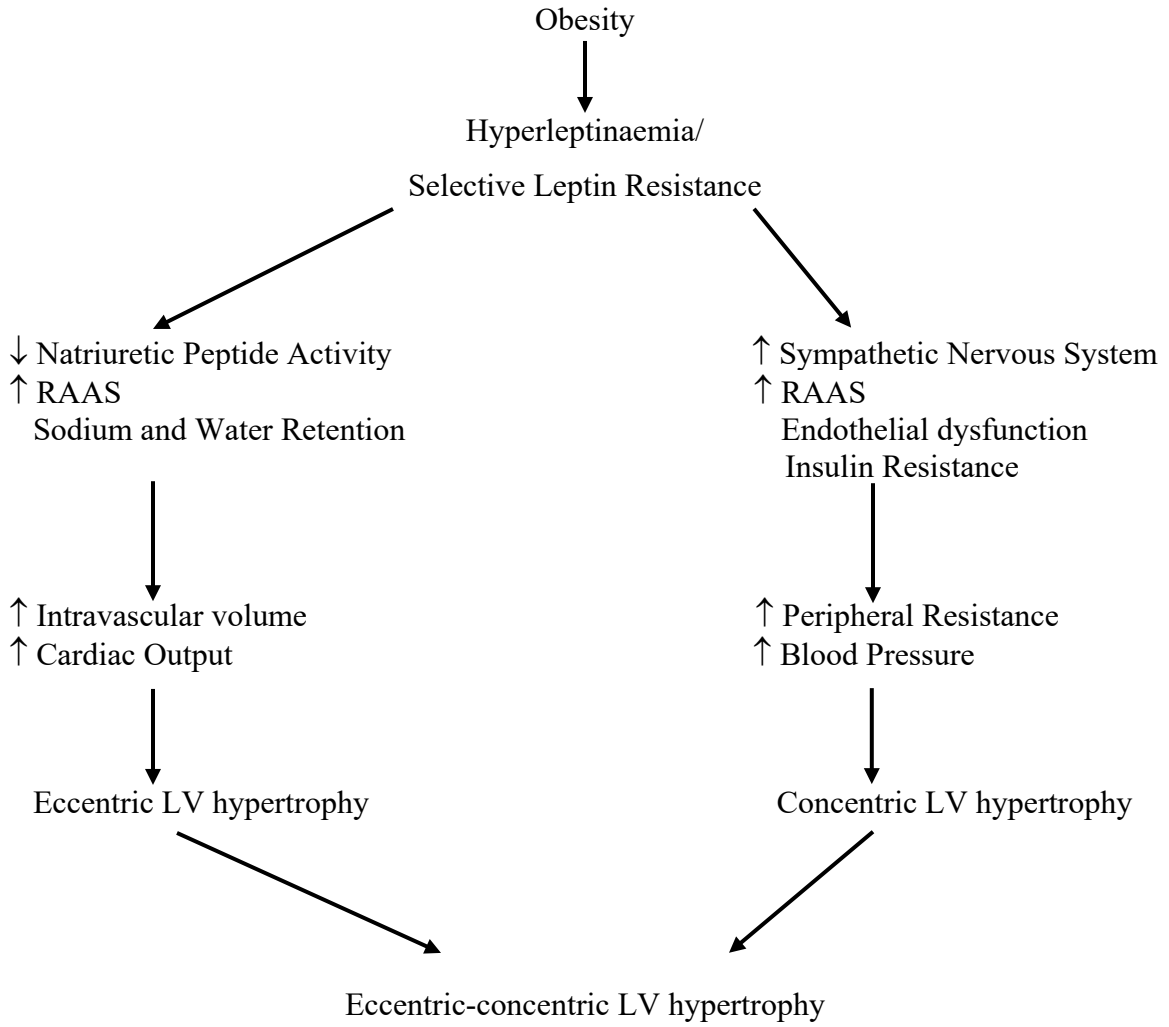


Figure 5 Flow chart outlining the role of Leptin in the development of LVH

Obesity is known to increase insulin resistance, with excessive nutrient intake, overactivation of the RAAS and hyperleptinaemia all implicated (219-221). Obesity therefore may also contribute to the development of LVH due to its close relationship with insulin resistance resulting in altered lipid and glucose metabolism with subsequent glucotoxicity and lipotoxicity and again increased oxidative stress (219). Insulin resistance will be discussed in more detail in the next section.

Finally obesity is associated with the increased formation of free radicals and oxidative stress (20). This is partly because adipocytes have been shown to produce numerous proinflammatory cytokines, such as TNF-α, IL-1 and IL-6, indeed obesity is considered a state of chronic inflammation (222). As discussed above obesity is associated with overactivation of the RAAS and angiotensin II has been shown to stimulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity which contributes a major source of reactive oxidative stress (ROS production in adipocytes (223). Obesity increases the mechanical load and therefore myocardial metabolism resulting in increased oxygen consumption. One effect of this is the production of increased free radicals (224).

This increased free radical production is important as there is substantial evidence that implicates oxidative stress with LVH and adverse cardiac remodelling and this will be discussed in more detail later (225).

1.3.4 Hyperinsulinaemia and Insulin Resistance

As discussed above LVH is common in people with diabetes. Obesity and hypertension are more common in patients with type 2 diabetes and therefore LVH may be more common in diabetes because of the close relationship with hypertension and obesity. However, LVH has been shown to develop independently of BP in subjects with diabetes (17). Insulin resistance has therefore been implicated in the pathogenesis of non-hypertensive LVH.

As discussed earlier biopsy specimens of humans with diabetic cardiomyopathy demonstrate a number of morphological abnormalities in addition to myocyte hypertrophy these include perivascular fibrosis, increased quantities of matrix collagen, cellular triglyceride and cell membrane lipid. These findings are consistent with the non-enzymatic glycation of vascular and membrane proteins, increased fatty acid uptake and hyperglycaemia-induced oxidative stress, which are characteristic of the diabetes state which is first and foremost disorder of abnormal insulin secretion and/or impaired insulin action (226).

Studies have demonstrated that higher plasma insulin concentrations are associated with increased LVM. Verdecchia et al (1999) studied 101 never treated non-diabetic patients with hypertension to determine whether insulin and insulin like growth factor (IGF-1) were independent determinants of LVM (227). Following multivariate analysis they demonstrated that IGF-1 and insulin accounted for over 40% of variability of LVM. This suggests that insulin and IGF-1 were powerful, independent predictors of LVM. A year later, Hirayama et al showed similar findings in 42 normotensive T2D patients with raised LVM. LVMI to BSA did not significantly correlate with plasma glucose and $HbA1_C$ but significantly correlated with plasma insulin concentrations (228).

Diabetes is associated with hyperglycaemia, hyperinsulinaemia and hyperlipidaemia. High concentrations of insulin are known to directly induce cardiomyocyte hypertrophy via the insulin receptor, Erk1/2. Erk1/2 is one 3 important MAPK subfamilies that regulate cardiac growth and hypertrophy (229, 230). In addition to this insulin resistance is associated with many cellular metabolic disturbances including reduced glucose metabolism and enhanced fatty acid metabolism which are believed to contribute to diabetic cardiomyopathy (230). An overview of these additional potential mechanisms by which insulin resistance is associated with LVH is discussed below and summarised in Figure 6.

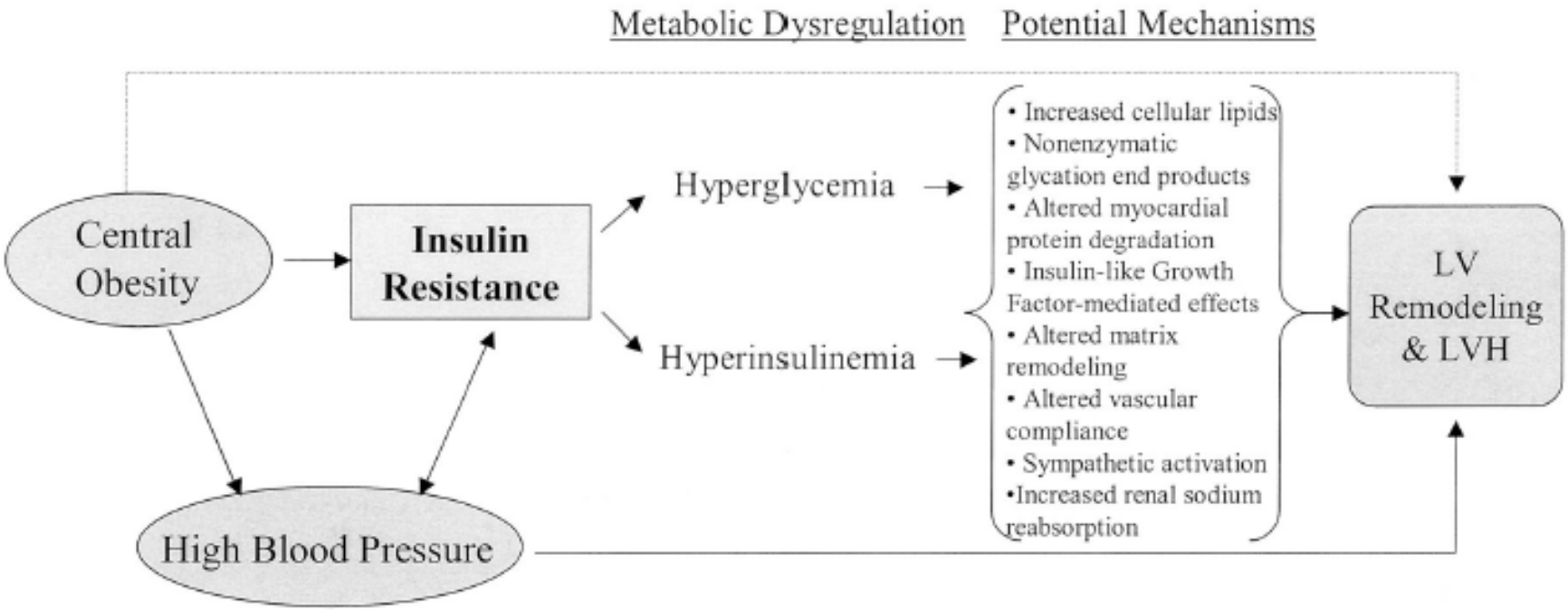


Figure 6 Potential mechanisms by which insulin resistance may cause LV remodelling and LVH (231)

Glucose is the primary fuel used by the foetal heart. At birth increased dietary fatty acids result not only in increased availability of fatty acid as fuel but the activation of the genes involved in fatty acid metabolism (232). In health the myocardium can switch between fatty acid and glucose fuel source depending on substrate availability which is referred to as metabolic flexibility (233). In patients with diabetes this flexibility is impaired. In diabetes given the presence of increased circulating triacylglycerol concentrations and insulin resistance (therefore reduced inhibition of lipolysis) there is enhanced delivery of fatty acids to cardiomyocytes. This leads to enhanced fatty acid β-oxidation and therefore the diabetic heart increases its reliance on fatty acids as a fuel (232). Furthermore fatty acids are known to inhibit glucose oxidation firstly through the Randle phenomenon and then later through activation of peroxisome proliferator-activated receptor α (PPAR α), a nuclear receptor that regulates cellar fatty acid metabolism (233, 234). Lipid metabolites are also known to impair insulin metabolic signalling contributing to insulin resistance and inhibit glucose uptake further exacerbating the reliance on fatty acid metabolism (235). Figure 7 summarises how fatty acid acids regulate glucose metabolism.

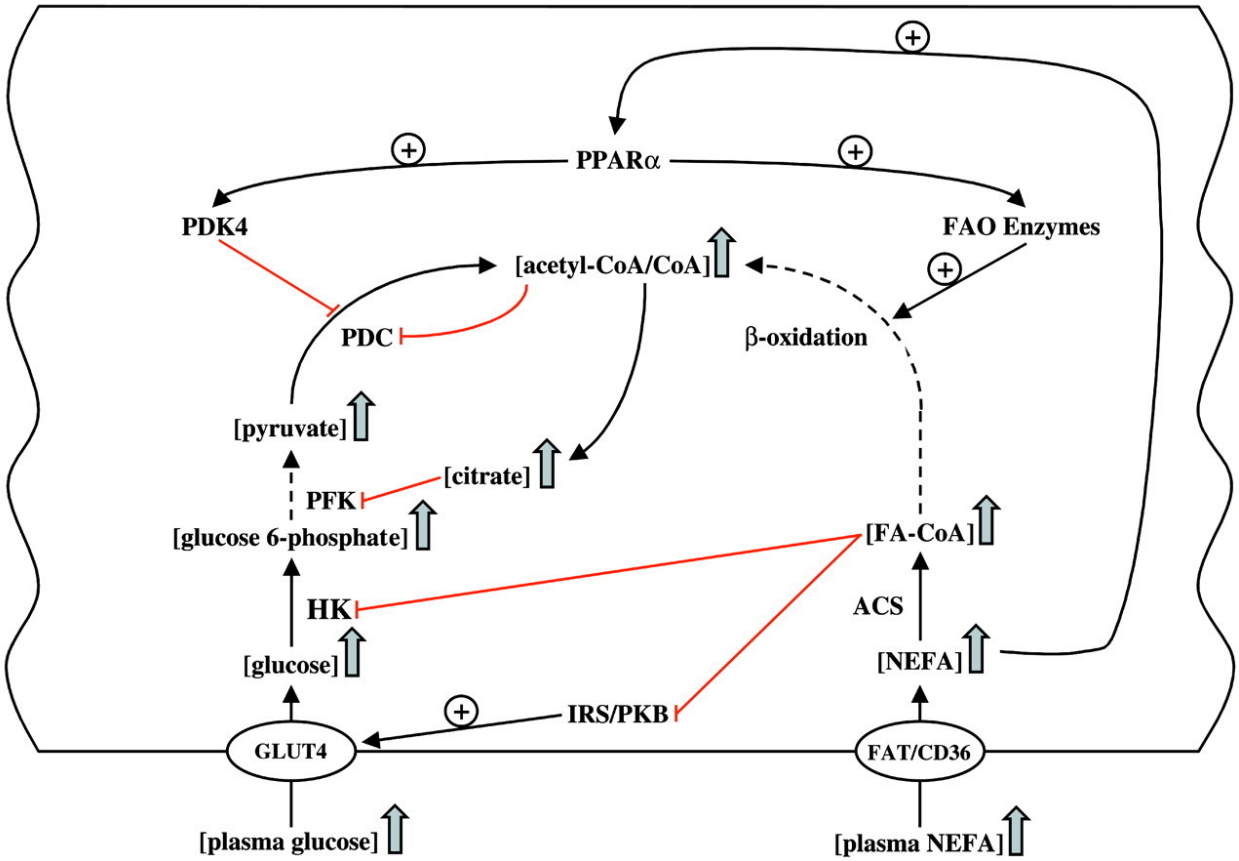


Figure 7 How fatty acids regulate glucose metabolism (230)

Abbreviations: FATCD36= Fatty Acid Translocase CD36, FAO = Fatty Acid Oxidation, HK = Hexokinase, IRS = Insulin Receptor Substrate, NEFA = Non Esterified Fatty Acid, PDC = Pyruvate Dehydrogenase Complex, PDK 4 = Pyruvate Dehydrogenase Kinase 4, PFK = Phosphofructokinase, PKB = Protein Kinase B, PPAR = Peroxisome Proliferator-Activated Receptor α.

Increased fatty acid oxidation is accompanied by increased generation of ROS which play a critical role in several pathways in the development of diabetic cardiomyopathy and specifically LVH (236). Furthermore, with excessive fatty acid availability, when the rate of fatty acid transport into the diabetic myocyte exceeds that of transport into the mitochondria, lipid metabolites, such as dicyclglycerol and ceramide begin to accumulate which lead to lipotoxicity. These cardiotoxic lipid intermediates reduce mitochondrial calcium uptake and induce mitochondrial permeability transition (MPT) pore opening further promoting mitochondria dysfunction and impaired ATP production resulting in caspase-mediated cell death/apoptosis and cardiac dysfunction (237).

The metabolic inflexibility of the diabetic heart also renders it vulnerable to hypoxic conditions such as times of ischaemia or increased workload such as pressure overload (e.g hypertension). In a healthy heart in such conditions myocardial substrate oxidation switches from fat to carbohydrate as glucose is a more oxygen-efficient fuel compared with fatty acids (238). Therefore, it is conceivable that an increased reliance on free fatty acids relative to glucose as in the setting of insulin resistance results in a decrease in cardiac efficiency and an increased susceptibility to heart failure.

Despite decreased glucose transporter expression secondary to insulin resistance in the diabetic environment the rates of glucose uptake in the diabetic heart are comparable to those observed in normal hearts due to hyperglycaemia (238). Therefore, with normal glucose influx into the cell and the inhibition of glycolysis secondary to an abundance of free fatty acids, glucose metabolites accumulate. This is further exacerbated by the fact that the inhibition of glycolysis by fatty acid oxidation is greater than the inhibition of glucose uptake (239).

The accumulation of glucose metabolites has many knock on consequences which contribute to diabetic cardiomyopathy and LVH and are summarised in Figure 8.

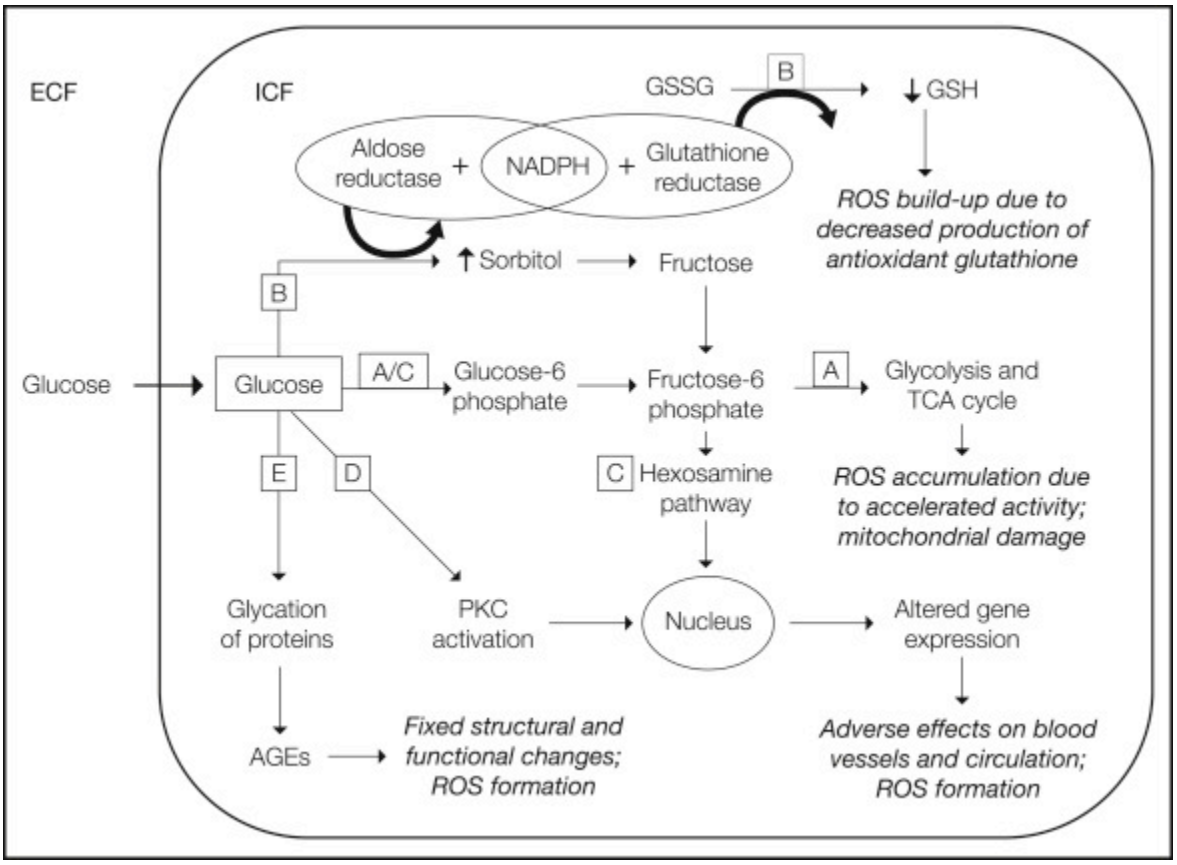


Figure 8 Metabolic Pathways of tissue damage resulting from glucotoxicity (240)

AGE = advanced glycation end product; ECF = extracellular fluid; GSSG = oxidized glutathione (which is reduced to the antioxidant GSH form); ICF = intracellular fluid; NADPH = nicotinamide adenine dinucleotide phosphate ; PKC = protein kinase C ; ROS = reactive oxygen species; TCA = tricarboxylic acid

Production of sorbitol is increased depleting NADPH and therefore limiting the production of the antioxidant glutathione which contributes to reactive oxidative stress (241). Some of the excess glucose enters the hexosamine pathway resulting in increases in advanced glycation end products (AGEs) such as N-acetylglucosamine (241). AGEs contribute to increased connective tissue crosslinking, fibrosis, cardiac stiffness and therefore LVH and impaired diastolic relaxation (242). They are also involved in the production of ROS further contributing to the development of oxidative stress and subsequent inflammation and fibrosis noted in diabetic cardiomyopathy (242). AGEs such as N-acetylglucosamine result in the O-linked glycosylation of various target proteins (243). Such target proteins include those involved in insulin signal transduction which may explain

why excessive glucose uptake is known to induce insulin resistance (244). Other proteins known to be activated by O-linked glycosylation include the transcription factors c-myc and Sp1 (245, 246). This is believed to lead to subsequent transcriptional adaption of the heart with induction of MHCβ and skeletal α-actin genes and concomitant repression of MHCα gene (247). This gene expression pattern is also noted in foetal and hypertrophied/overloaded hearts suggesting a common signal must be present (247). The common factor is most likely glucose. Indeed the induction of skeletal α-actin is known to be dependent of Sp1 activation which is known to be activated in response to increased glucose availability in the liver (246). Glucose is also the primary fuel of the foetal heart and during pressure overload when it is known that the heart increases its reliance on glucose (248, 249). The higher rate of glucose uptake will exceed pyruvate oxidation resulting once again in the accumulation of glycolytic intermediates leading to the MHC isoform switch described above in diabetic hearts. It is therefore clear that metabolism is not an innocent bystander when it comes to gene expression in the heart. Indeed, it seems that glucose sensing appears crucial in the adaption of the myocardium to various stimuli whether they are haemodynamic (e.g. increased workload) or metabolic (e.g. diabetes).

Such adaption however may become detrimental. In the same way that fatty acids are able to inhibit the utilisation of glucose, conversely glucose is able to inhibit fatty oxidation in the heart (250, 251). Glucose oxidation produces citrate which can be converted into malonyl-CoA and elevated intracellular levels of malonyl-CoA are known to inhibit the beta-oxidation of fatty acids (251). There is also evidence that glucose exposure directly decreases the expression of fatty acid metabolising genes such as PPARα (252).

Therefore in the setting of increased glucose oxidation such as if diabetes progresses (persistent hyperglycaemia) or additional stresses are placed on the heart (e.g. hypertension) metabolic maladaptation may occur. Decreased PPARα expression due to this pressure overload or prolonged exposure to hyperglycaemia will limit the fatty acid oxidation of the heart increasing lipid metabolite accumulation accelerating cardiac dysfunction secondary to lipotoxicity. This metabolic adaption and maladaptation is depicted in Figure 9 (232).

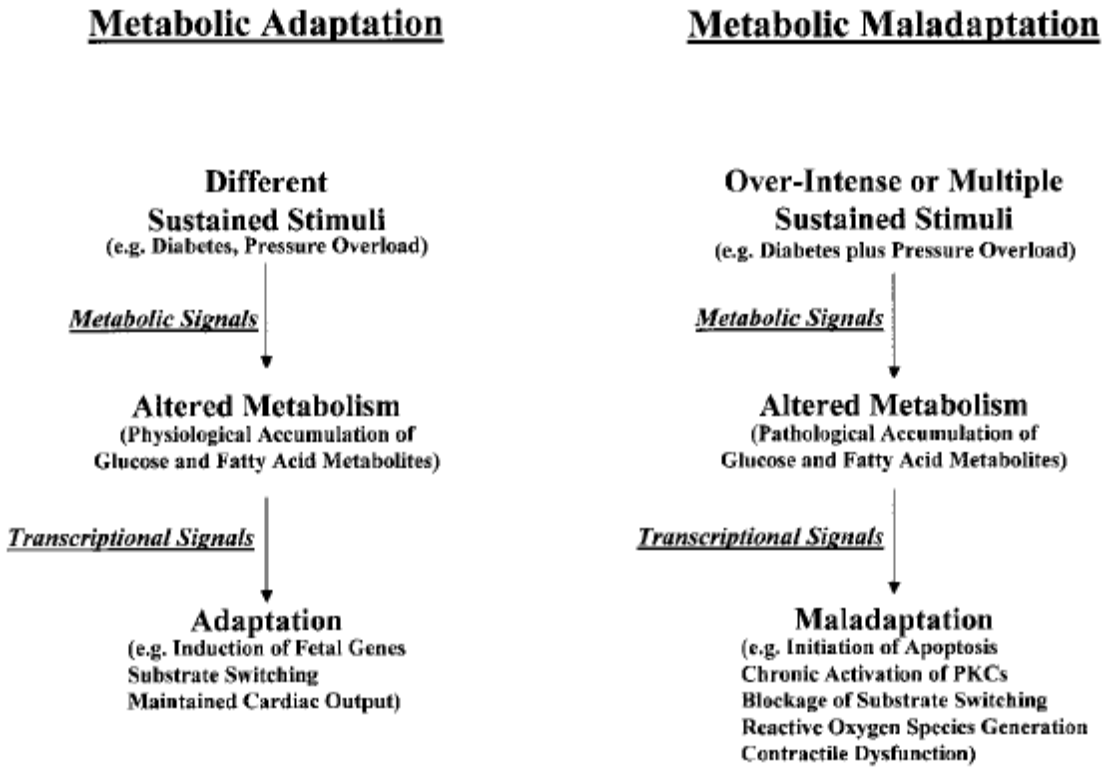


Figure 9 Metabolic adaptation and maladaptation to various haemodynamic and metabolic stimuli (232)

1.3.5 Reactive Oxidative Stress

1.3.5.1 The Concept of Oxidative Stress

Reactive oxidative species is a collective term for the by-products of the oxygen metabolism characterised by their high reactivity. They include free radicals (i.e. species with greater than one unpaired electrons such as hydroxyl (OH^-) and superoxide (O_2^-) non-radicals capable of generating free radicals such as hydrogen peroxide (H_2O_2) (225). O_2^- is formed by mitochondrial respiration or by various enzymes including NAD(P)H oxidase, NO synthase and xanthine oxidase (XO) (175). In health the levels of ROS are maintained by an antioxidant defence system primarily made up of two enzymes superoxide dismutase (SOD) and catalase. These enzymes scavenge and degrade ROS to inactive molecules. The balance between ROS production and their removal by antioxidant system is known as the redox state of a cell. Oxidative stress in the term used when

there is a pathological imbalance in favour of excess ROS. ROS and the enzymes that regulate their levels is summarises in Figure 10(175).

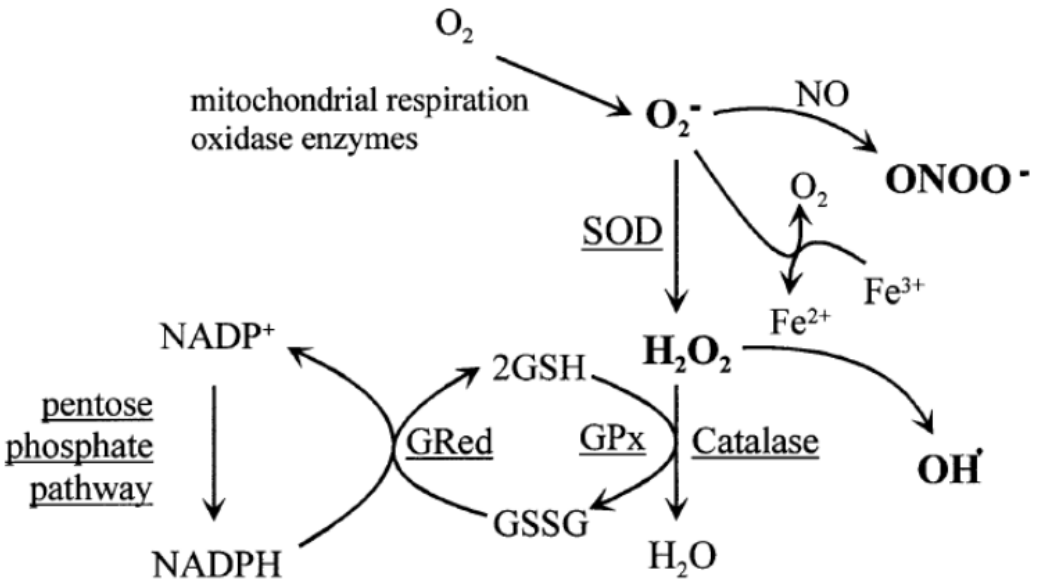


Figure 10 Reactive oxidative stress and the enzymes that regulate their levels (175)

The two main sources of ROS in LVH and heart failure are XO and NADPH oxidase (225). XO expression and activity are increased in experimental models of CHF and XO related oxidative stress is found in pressure overload of the heart (253, 254). NADPH oxidase activity has also been found to be increased in experimental models of LVH and heart failure (255-257).

In excess, ROS can induce oxidation and damage to proteins, membranes, DNA, RNA and other macromolecules and is involved in the pathophysiology of a range of diseases such as atherosclerosis, renal disease and cancer (225). Increased oxidative stress is implicated in most types of chronic heart failure, including that from ischaemic, non-ischaemic cardiomyopathy including diabetic and hypertensive cardiomyopathy (pressure overload) (258). This is perhaps not surprising as most of the important processes underlying cardiomyopathy such as inflammation, angiogenesis, hypertrophy/apoptosis, fibrosis and contractile dysfunction are redox sensitive (259).

1.3.5.2 Working Model of How Reactive Oxidative Stress Causes Left Ventricular Hypertrophy

Traditionally it has been believed that ROS caused its pathological effects simply by free radical-induced oxidation and damage, resulting in mitochondrial and cell dysfunction, necrosis and apoptosis. More recently however, it is believed that ROS also modulate intracellular signalling pathways and proteins (225). Indeed low levels of ROS are thought to play a role in normal cardiac signalling, growth adaptions and matrix changes (253). Higher levels however are believed to play a role in pathophysiologic remodelling which is relevant not only to chronic heart failure but also to predisposing conditions such as LVH (253).

ROS are thought to activate a variety of hypertrophy signalling kinases leading to the development and perpetuation of myocyte hypertrophy. They also appear to mediate the hypertrophic response to other known hypertrophic stimuli we have described including mechanical strain, angiotensin II and α-adrenergic stimulation (175). Furthermore, mechanical stretch can induce the release of preformed Angiotensin II which increases the production of ROS via the stimulation of NADPH oxidase (260). A vicious cycle therefore ensues in that pressure overload produces oxidative stress which further exacerbates the hypertrophic response to the pressure overload. This is summarised in Figure 11 (175).

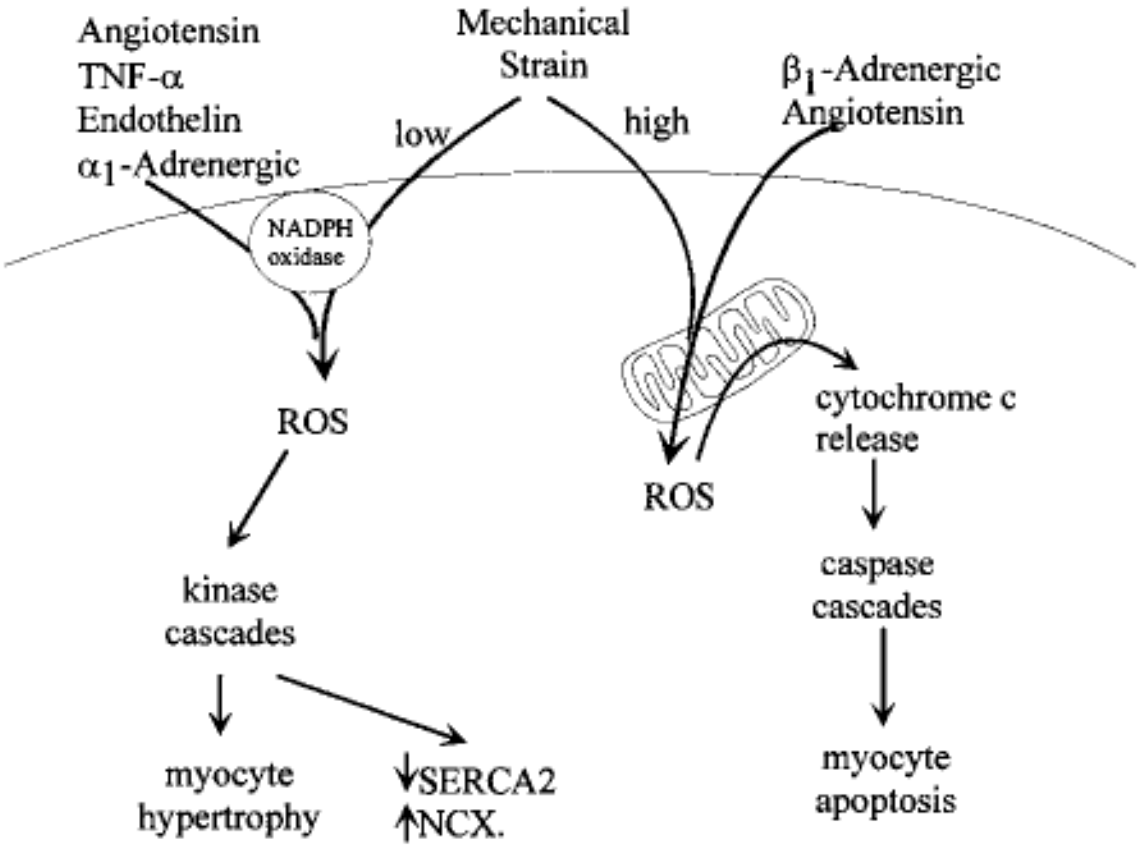


Figure 11 Reactive oxidative stress and how they may mediate the hypertrophic and atrophic effects of several stimuli on cardiac myocytes (175)

Central to the ROS mediation of the hypertrophic response to a variety of hypertrophic signals depicted in figure 12 appears to be the activation of the Na^+/H^+ exchanger (NHE-1) (260). Indeed, it is thought that NHE-1 activation represents a common response to mechanical stretch and a key player in the hypertrophic response. NHE-1 is an integral membrane glycoprotein expressed in mammalian cells and it is electroneutral exchanging intracellular H^+ for extracellular Na^+ to regulate intracellular Ph (260). Activation of the NHE is increasingly documented as a process involved in cardiac hypertrophy and heart failure (261). It is believed that increased ROS such as secondary to increased Angiotensin II and endothelin stimulation activates the ERK 1/2p90 pathway resulting in upregulation in the NHE-1 exchanger (260). This results in increased intracellular Na^+ and this increase results in an increased intracellular Ca^{2+} due to reduced Ca^{2+} efflux through the Na^+/Ca^{2+} exchanger (NCX) as summarised in Figure 12 (260)

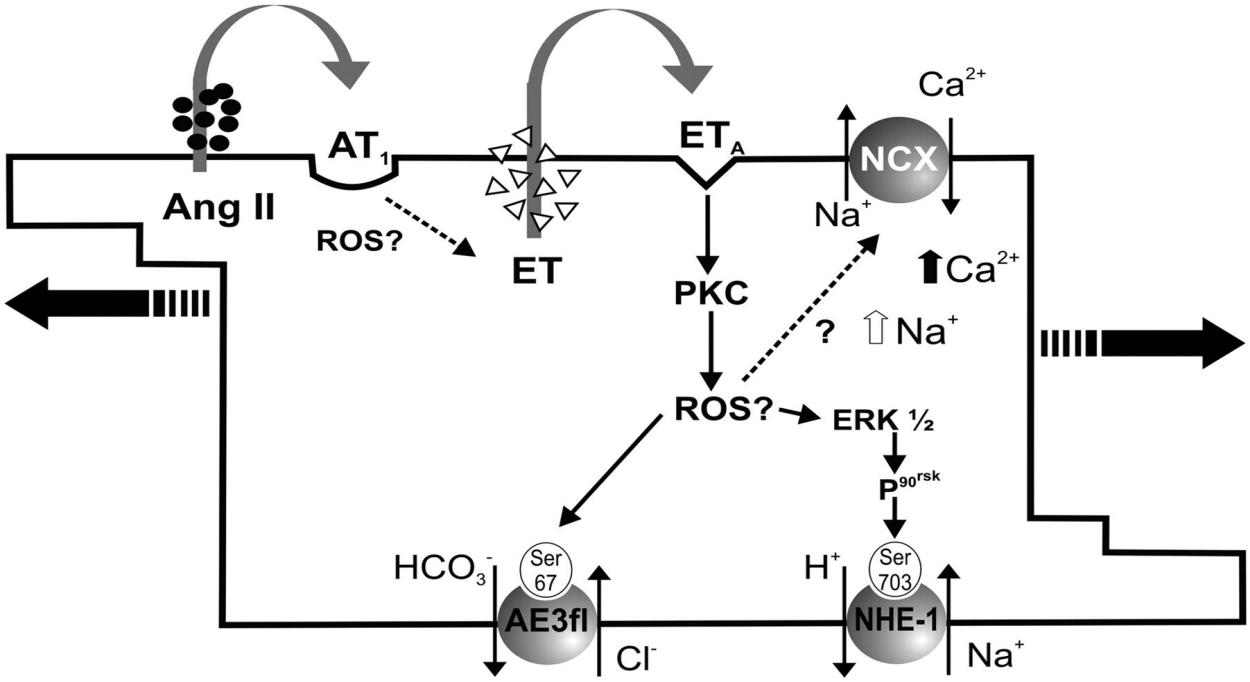


Figure 12 The role of the Na+/H+ exchanger in the ROS mediation of the hypertrophic response to hypertrophic signals (260)

Strong evidence now exists that the increased intracellular Na^+ and Ca^{2+} levels seen with hyperactivity of NHE-1 are implicated in myocardial dysfunction, hypertrophy and apoptosis (262). Intracellular Na^+ and Ca^{2+} loading has been observed in diabetic hearts, particularly in heart failure (263-265). Reduction of myocardial intracellular Na^+ concentration by inhibition of Na^+/Ca^{2+} or Na^+/H^+ exchangers has also been shown to improve heart failure and cardiac hypertrophy (262, 266). Such findings further implicate the NHE-1 as a major factor in the cardiac remodelling process regardless of the initiating factor.

Despite the emerging role for NHE-1 involvement in cardiac remodelling the mechanisms underlying this role remain unclear. Theories suggested are that the increased levels of intracellular Na^+ activates various protein kinase C (PKC) isoforms which then alters gene expression and protein synthesis (267). The increased levels of intracellular calcium may activate pro-hypertrophic factors such as calcinuerin. In addition to this, calcium overload in cells particularly when accompanied by oxidative stress increases the MPT pores. MPT pore opening is detrimental to mitochondria resulting in mitochondrial dysfunction, impaired ATP synthesis, increased ROS and cell death/apoptosis (267). All these factors may combine to contribute to activation of transcriptional factors resulting in cardiac hypertrophy as summarised in Figure 13 .

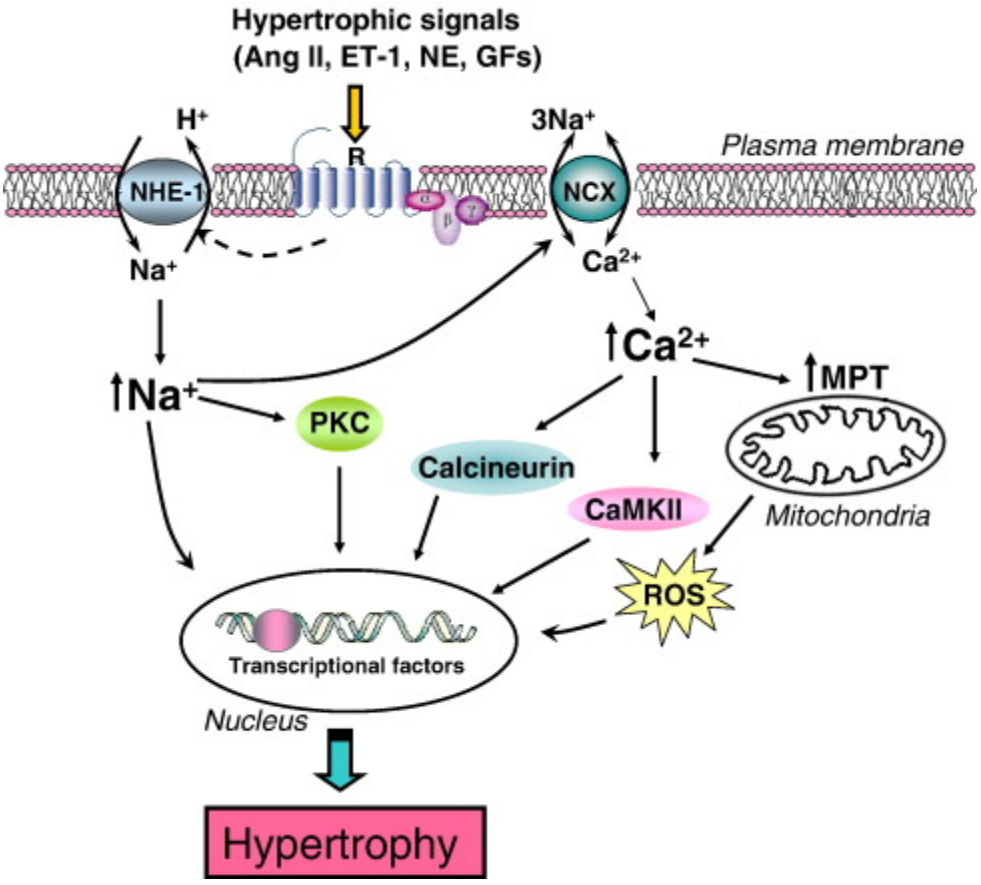


Figure 13 Possible mechanisms by which the NHE-1 exchanger promotes LVH (267)

Either way the general consensus is that the NHE-1 activated by ROS plays a major role in regulating cell growth and may indeed be a common downstream mediator for the hypertrophic effect of mechanical strain and a significant number of hormonal, paracrine and autocrine factors. As a consequence NHE-1 has become a highly attractive therapeutic target for cardiac hypertrophy and heart failure.

ROS also known to have potent effects on the extracellular matrix, promoting cardiac fibroblast proliferation and activating matrix metalloproteinases (MMPs) which are central to fibrosis and matrix remodelling (268). MMPs are secreted in an active form but are activated post translationally by ROS and ROS are known to stimulate transcription factors such as activator protein-1 that increases MMP expression (255).

ROS is also thought to directly influence contractile function of the myocardium by modifying proteins crucial to excitation-contraction coupling (253). Among all cardiac ion transporters and channels the ryanodine receptor complex appears to be the most sensitive to redox modification

(269). Calcium release through the ryanodine receptors (RyR2) is essential for initiating a robust myocardial contraction. Elevated ROS has been shown to lead to increased RyR2 oxidation and therefore irregular calcium handling resulting in contractile dysfunction in failing hearts (270)

Increased ROS is also important in the development of vascular endothelial dysfunction which contributes to systemic vasoconstriction and increased cardiac loading. This is primarily due to the reduction in NO bioavailability (225).

The main pathophysiological effects of oxidative stress in heart failure and LVH are summarised in Figure 14 (225).

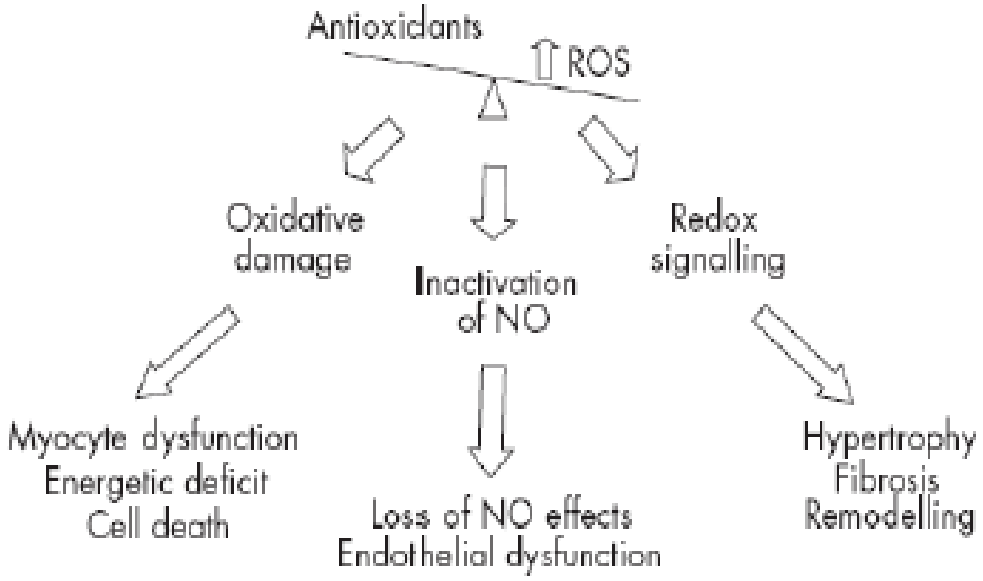


Figure 14 Pathophysiological effects of oxidative stress in LVH and heart failure (225)

1.3.5.3 Diabetes as a Source of Oxidative Stress

Patients with diabetes are known to have increased oxidative stress. In T2D this is primarily driven by increased mitochondrial ROS (especially O_2^-)(258). This occurs via a number of mechanisms ultimately secondary to the increased cellular flux of glucose and fatty acids as described in detail in the previous section. The inappropriate activation of RAAS is also implicated as discussed below.

Indeed, whilst hyperglycaemia regulates multiple pathways in the diabetic heart, increased ROS generation and depleted anti-oxidant defence ultimately result in oxidative stress is thought to represent a central mechanism underlying the adverse cardiac remodelling seen in the diabetic heart. Therefore, ROS appears crucial to many of the micro- and macro-vascular complications in diabetes including LVH and heart failure.

1.3.6 Renin Angiotensin Aldosterone System

Increased activation of both systemic and local RAAS despite a state of sodium and volume excess in states of insulin resistance is well established (219). Serum angiotensin II levels are significantly correlated with post proprandial glucose concentrations in insulin resistance and T2D (271). In addition to this, the pro-inflammatory angiotensin II receptor 1 is upregulated and the anti-inflammatory AT-2R is downregulated in early diabetes mellitus (272).

The overactivation of RAAS, in particular the elevated levels of angiotensin II contribute to the development of LVH in a number of ways. Firstly, RAAS promotes the retention of sodium and water and angiotensin II causes vasoconstriction and therefore increased vascular resistance and arterial BP (273). Secondly, angiotensin II has direct effects on cardiomyocytes and cardiac fibroblasts through AT1 receptors promoting cardiac hypertrophy and fibrosis (274). Thirdly, activation of the RAAS also contributes to systemic and cardiac insulin resistance through the mTOR-S6K1 signal transduction pathway (275). Finally both angiotensin II and aldosterone directly prompt oxidative stress by increasing NADPH oxidase (276). As discussed above insulin resistance and reactive oxidative stress are both heavily implicated in the development of LVH.

1.3.7 Genetics

As discussed above, hypertension, and obesity often co-exist with T2D contributing to LVH development. T2D is also independently associated with LVH when other covariates such as age, BMI and hypertension are accounted. However, not all patients with T2D develop LVH and those developing LVH do so to varying degrees. This heterogeneity is most likely secondary to a partial genetic basis. Indeed, it has been estimated that 30% of LVH has a genetic component (277). As with most CV traits of interest including the susceptibility to heart disease such as hypertension the link between genetic predisposition and LVH is described as a “complex trait”. In other words

unlike Mendelian traits there is not a simple one to one relationship between genotype and phenotype (278). Therefore, genetic studies of multifactorial disorders such as LVH are challenging.

However, LV mass heritability in non-diabetic subjects has been estimated through twin studies, studies in hypertensive siblings and through complex family studies (279-282).

Two population-based genome wide association studies (GWAS) have attempted to identify single nucleotide polymorphisms (SNPs) associated with LVH in the general population (283, 284). Vasan et al, estimated the association between 2.5 million SNPs and echocardiographic traits including LVH. They found 2 SNPs associated with increased LV mass and 3 SNPs associated with increased LV wall thickness at genome wide significance. However, these SNPs were not replicated in the independent sample (283). Shah et al, reviewed 3 population-based cohorts: The British Women's Heart and Health Study (BWHHS, n=3443), the Genetic Regulation of Arterial Pressure of Humans In the Community (GRAPHIC) Study (n=2024) and the Whitehall II Study (WHII) (n=5059). They found 4 SNPs associated with ECG LVH with genome wide significance which were also replicated in the independent cohort (284).

Parry et al, were the first to carry out a genetics study investigating LVH in T2D when they attempted to identify genetic variants predicting LVH in diabetic individuals (285). In a GWAS of a large population of patients with T2D in Tayside, two out of the nine previously identified SNPs associated with LVH were again found to be significant. These included rs17132261 which is found near the SLC25A46 gene that codes for a mitochondrial phosphate transporter (286). Given the above discussion about the potential importance of myocardial energetics in the pathogenesis of diabetic cardiomyopathy it may be logical that variation here be associated with LVH. The second replicated SNP, rs2292462 is found in the NMB gene, which is believed to be associated with satiety and weight regulation (287). The variation in rs2292462 was not predictive however of obesity in the study when weight was accounted for. This implies that the association with LVH is not driven by its link with obesity (285).

Clearly if specific genes known to lead to LVH could be identified this could improve risk stratification in diabetic patients so intensive preventative measures could be taken. In addition to this, it would open up a potential for new molecular drug targets although more research is required.

1.4 Treating Left Ventricular Hypertrophy

We now know that LVH is associated with increased risk of mortality and morbidity several-fold above the risk due to hypertension alone. LVH, can be targeted by BP reduction and lifestyle modifications such as weight loss and dietary sodium restriction. It is known that despite the substantial benefits of BP reduction, conventional treatment does not normalise the risk of CV events in patients with hypertension. This raises many questions, does LVH regression result in reduced mortality and morbidity beyond simply lowering BP? Does the choice of antihypertensive medication matter and if so why? Should we be focusing on downstream pathways and oxidative stress given their role in the pathogenesis of LVH?
These questions will be discussed below.

1.4.1 Benefits of Regressing Left Ventricular Hypertrophy

Regression of LVH has consistently been shown to be associated with improved prognosis. Levy et al followed up subjects from the Framingham Heart Study with ECG LVH defined using the Cornell voltage criteria (288). Subjects with a serial decline in voltage were at lower risk for CVD than were those with no serial change (men odds ratio 0.46; 95% CI, 0.26 to 0.84; women: odds ratio, 0.56; 95% CI, 0.30 to 1.04) after adjusting for age and baseline voltage. In contrast, those with a serial increase in voltage were at greater risk for CVD (men: odds ratio, 1.86; 95% CI, 1.14 to 3.03; women: odds ratio, 1.61; 95% CI, 0.91 to 2.84).
As discussed above LVH defined by ECG criteria is less sensitive than echocardiography but the improvement in prognosis with LVH regression persists even when echocardiography is used to define LVM.
Verdecchia et al followed 430 patients with essential hypertension of which 26% had echo LVH with an LVMI of 125g/m^2 or greater (289). Patients were treated with various hypertensives and

lifestyle changes to maintain BP below 140/90. The patients with a decrease in LV mass from the baseline to follow-up visit were compared with those with an increase in LV mass. There were 15 CV events (1.78 per 100 person-years) in the group with a decrease in LV mass and 16 events (3.03 per 100 person-years) in the group with an increase in LV mass (p=.029). In the subset with LV mass greater than 125 g/m^2 at the baseline visit the event rate was lower among the subjects who achieved regression of LVH than in those who did not (1.58 versus 6.27 events per 100 person-years; p=.002). Importantly, this improvement in prognosis was independent of baseline LVM, BP and also the degree of BP reduction.

The same group confirmed these findings of an association between LVH regression and lower CV events with a meta-analysis of four trials (290). In total 1064 hypertensive patients were included in the analysis and it showed that LVH regression significantly reduced the risk of CV events (OR=0.41, 95% CI: 0.21–0.78, p = 0.007)

In addition to a reduction in CV mortality numerous other CV benefits with LVH regression have also been demonstrated with a reduced number of ventricular premature beats, decreased vulnerability to inducible ventricular fibrillation and less hospitalisation for heart failure in hypertensive patients (291-293).

Overall, it is clear that LVH regression has positive prognostic impact on CV morbidity and mortality independent of BP reduction.

1.4.2 Established and Potential Ways of Regressing Left Ventricular Hypertrophy

i. Anti-Hypertensive Therapy

So we now know that LVH regression is associated with better outcomes and primarily LVH can be targeted through BP reduction.

However, whilst all anti-hypertensive drugs appear to have some effect on LVH regression, antihypertensive drugs do not necessarily have equivalent effects on LVM. This is consistent with our understanding that additional factors modulate the expression of LVH and that the benefit of LVH regression is not entirely explained by BP control.

Klingbeil et al performed a meta-analysis of double-blind trials that measured the effect of antihypertensive therapy on LVM (172). In total 80 trials with 146 active treatment arms (n=3767 patients) and 17 placebo arms (n=346 patients) were included. After adjustment for treatment duration and diastolic BP they found a significant difference among the different medication classes (p=0.004). Overall, LVM index was decreased by 13% with angiotensin II receptor antagonists (95% CI: 8 – 18), by 11% with calcium channel antagonists (95% CI: 9 – 13), by 10% with ACE inhibitors (95% CI: 8 – 12), by 8% with diuretics (95% CI: 5 – 10), and by 6% with β-blockers (95% CI: 3 – 8). Another Meta-analysis performed by Schmieder et al also demonstrated that drugs targeting the RAAS appeared to be most potent in regressing LVH (294). This is perhaps non-surprising given that we know that activation of the RAAS causes hypertrophy of the cardiomyocyte similar to load-induced hypertrophy and fibrosis, both of which are mediated by angiotensin II (295).

The Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) and the Heart Outcomes Prevention Evaluation (HOPE) are two large landmark trials which have specifically looked at the effects of BP and LVM reduction with ARBs and ACE inhibitors.

The LIFE Study aimed to establish whether selective blocking of angiotensin II improved LVH beyond simply reducing BP and consequently reducing CV morbidity and death (296). In total 9193 participants aged 55-80 years old with hypertension and ECG LVH were included. Participants were given either a losartan based or atenolol based antihypertensive regimen and followed up for an average of 4.8 years for composite endpoint of CV death, fatal and nonfatal MI and fatal or nonfatal stroke. BP reduction was similar in both treatment arms. Despite this the losartan based antihypertensive regimen resulted in greater reduction in LVMI from baseline compared with the atenolol regimen (-21.7+/-21.8 vs -17.7+/-19.6g/m^2, p=0.021) This reduction was independent of the baseline LVM index and BP. This greater LVM reduction correlated with improved outcomes with the primary endpoint occurring less in the losartan treatment arm. The primary composite endpoint occurred in 508 losartan (23.8 per 1000 patient-years) and 588 atenolol patients (27.9 per 1000 patient-years; RR0.87, 95% CI 0.77-0.98, p=0.021). Losartan was better than atenolol in terms of CV mortality with 204 losartan and 234 atenolol patients dying from CVD (0.89, 0.73-1.07, p=0.206); 232 and 309, respectively, had fatal or non-fatal stroke (0.75, 0.63-0.89, p=0.001); and MI (non-fatal and fatal) occurred in 198 and 188, respectively (1.07, 0.88-1.31, p=0.491).

Interestingly and never fully accounted for, the incidence for new-onset diabetes was less frequent with losartan by 25% (296).

The onset of diabetes was studied in more depth at a later date by Okin et al who looked into the onset of diabetes in a sub study of the LIFE study (297). In total 7,998 LIFE patients without diabetes were followed to see who developed diabetes and also underwent serial ECGs. They observed that resolution or absence of ECG LVH criteria was associated with a lower incidence of diabetes, even after adjusting for losartan and other risk factors for diabetes.

Sub-group analysis of 1,195 patients in the LIFE trial, revealed that, as in the whole trial analysis, losartan was also more effective than atenolol at reducing CV morbidity and mortality in patients with diabetes, ECG LVH and hypertension (298). However, the regression of LVH did not occur to the same extent in patients with diabetes despite similar BP reduction in comparison to non-diabetic patients (299). This helps to explain why despite BP treatment, patients with diabetes still fair worse in terms of CV morbidity and mortality.

The (HOPE) trial aimed to evaluate the benefit of ACE inhibition in high risk patients with ECG LVH (300). In total 9,297 without heart failure were included and randomly assigned to either ramipril or placebo which was added to their concomitant medications which in a substantial proportion of patients included antihypertensive drugs (excluding ACEi). As a result, despite a history of hypertension in nearly 50% of the participants and unlike in many trials in hypertension the baseline BP in the HOPE trial was normal or near normal. ECGs were performed at baseline and at the end of the study and patient. The reduction in BP attributable to ramipril was modest (a fall of 3-4 mmHg systolic BP and 1-2 mmHg diastolic). Despite this substantial benefit were observed in the Ramipril arm, indeed the trial was stopped early because of the convincing evidence of the benefit of ramipril on the combined primary endpoint of MI, stroke or death from CV causes. The primary endpoint was reached in 14% of the Ramipril group and 17.8% in the placebo group resulting in a RR reduction 0.78 (95% CI 0.70 – 0.86, $p < 0.001$). This comprised a risk reduction of 32% for stroke, 20% for MI, 26% for CV death and 16% for all-cause mortality,

Significantly fewer patients in the ramipril group had a cardiac arrest, worsening angina, heart failure or a new diagnosis of diabetes. These differences were seen after correcting for diabetes, age, and most importantly hypertension.

With regards LVH at baseline, 676 patients had LVH (321 in the ramipril group and 355 in the placebo group) and 7605 patients did not have LVH (3814 in the ramipril group and 3791 in the placebo group). The study patients treated with Ramipril were protected against LVH and regression of LVH was independent of BP with ramipril treatment. At the conclusion of the study, 336 patients in the ramipril group (8.1%) compared with 406 in the placebo group (9.8%) had development/persistence of LVH; in contrast, 3799 patients in the ramipril group (91.9%) compared with 3740 in the placebo group (90.2%) had regression/prevention of LVH (p=0.007). Patients who had regression/prevention of LVH had a lower risk of the predefined primary outcome (CV death, MI, or stroke) compared with those who had development/persistence of LVH (12.3% versus 15.8%, p=0.006) and of chronic heart failure (9.3% versus 15.4%, p<0.0001).

The reduction in events particularly for MI in the HOPE trial was much greater than would have been expected from such a modest fall in BP observed in the ramipril arm. It is thought with older anti-hypertensive agents that there is an approximate 38% reduction in stroke and a 16% reduction in MI when diastolic BP is reduced by 4-5mmHg over a period of 4-5 years. This was achieved in the HOPE trial with much more modest reduction of only 1-2mmHg. In addition to this the HOPE trial as alluded to above was unique in that the baseline BP was normal or near normal. This suggests that there are benefits in LVM reduction even in patients with BP within the "normal" range. Sub studies of the HOPE trial have specifically investigated the effects of ramipril on LVM in patients with controlled BP (301). Lonn et al randomised 506 patients with a normal BP (baseline average 131/76) to either ramipril (10mg/day or 2.5mg/day) or placebo. After four years LVMI increased by 3.98 +/- 2.08 g/m^2 in the placebo group and by 4.16 +/- 1.86 g/m^2 in the ramipril 2.5 mg/day groups. LVMI decreased by 2.02 +/- 2.25 g/m^2 in the ramipril 10 mg/day group (p = 0.02). This suggests that ramipril reduces LVM by other means than just BP and is likely due to the inhibition of the angiotensin II effects on the myocardium discussed earlier.

The Effects of Ramipril on Cardiovascular and Micro-vascular Outcomes in People with Diabetes Mellitus (MICRO HOPE) study looked at the sub-group of patients with diabetes recruited in HOPE (n=3,577) (173). Ramipril significantly reduced the risk of the combined primary outcome of MI, stroke or CV death in diabetic patients by 25% (p=0.004). Again, the cardiovascular benefit

was greater than that attributable to the decrease in BP. Indeed, after adjustment for BP changes, the results remained significant.

Despite the clear benefit of RAAS inhibition it is not clear whether dual blockage of RAAS with different pharmacological agents leads to additional benefits in BP control and LVM reduction. The 4E-Left Ventricular Study was a double-blind randomised study, which studied LVH regression among groups treated with eplerenone, enalapril and eplerenone combined with enalapril (302). They were followed up for 9 months and changes in LVM were assessed by MRI. All three groups showed a significant decrease in LVM. The combined eplerenone and enalapril group was most effective in terms of absolute reduction in LV mass. However, the additional benefit with dual RAAS blockage has not been demonstrated in other studies. In the Ongoing Telmisartan Alone and in Combination with Ramipril Global End Point Trial (ONTARGET) the combination of ramipril and telmisartan had a similar effect on LVH as ramipril alone among the patients at high vascular risk (303). Similar findings were reported from Aliskiren in Left Ventricular Hypertrophy (ALLAY) trial (304). A total of 465 patients with hypertension, increased ventricular wall thickness were randomised to receive aliskiren 300 mg, losartan 100 mg, or their combination daily for 9 months. Cardiac MRI was used to assess LVM at the baseline and at completion of study. LV mass index was reduced significantly from baseline in all treatment groups (4.9, 4.8, and 5.8 g/m^2 reductions in the aliskiren, losartan, and combination arms, respectively; $p<0.0001$ for all treatment groups). However, there was not much difference between monotherapy and combination therapy independent of BP lowering.

In summary, it is clear that if a patient has ECG or echo LVH, then blockade of the RAAS system should be considered along with tight BP control. The benefits extend to people with diabetes and blockade of the RAAS also has beneficial effects on the development of new onset diabetes. This approach however is only partially effective as LVH persists in 20% of hypertensive patients who attain target BP (305). As controlling BP is only partially effective at regressing LVH, additional methods are required. Possible targets include oxidative stress and insulin resistance.

1.4.2.1 Glycaemic Control

The other therapeutic target whose manipulation might regress LVH is insulin resistance. Abnormal glucose metabolism is linked to insulin resistance and as discussed above there is several lines of evidence suggesting that there is a significant relationship between LVH and insulin resistance.

Aepfelbacher et al sought to determine whether improved glycaemic control in patients with T1D helps to regress LVH (306). In total 19 patients with T1D were included in a program of stringent glycaemic control and echocardiograms were performed at baseline and after 1 year. In the patients (n=12) with improved glycaemic control haemoglobin A1c decreased from 9.8% to 7.8% ($p< or =0.0001$), interventricular septal thickness decreased from 10.3 to 9.4 mm ($p< or =0.05$), and LVM regressed from 205 to 182 g ($p< or =0.05$). Septal thickness and LVM remained unchanged in the patients who did not achieve improvement of glycaemic control.

Felicio et al aimed to evaluate the influence of tight blood glucose control on LVM in patients with T2D (307). Fifty-six hypertensive patients and 26 healthy controls were included. The diabetic patients were followed up for one year and echocardiograms performed at baseline and at one year. In total, 10% of the participants were noted to have a regression in LVM of greater than 10% and in this group fasting blood glucose concentrations fell from 178+/-36 to 147 +/-30 mg/dl ($p<0.1$) and a correlation was observed between blood glucose and LVMI percent variations (delta) ($r=-.48$, $p<0.01$). This would suggest that the improvement in glycaemic control may contribute to LVH regression in hypertensive patients with T2D.

1.4.2.2 Oxidative Stress

More recently, there has been more interest in downstream pathways and oxidative stress in the pathogenesis of LVH. Allopurinol reduces tissues oxidative stress and as discussed above oxidative stress is a mediator of myocardial hypertrophy (175, 253, 308).

Many studies have also shown that allopurinol can regress LVH in various experimental models of cardiac disease (309) (310).

In a recent randomised double-blind, placebo-controlled study Szwejkowski et al demonstrated that allopurinol 600 mg/day reduced absolute LV mass (−2.65 ± 5.91 vs. + 1.21 ± 5.10 g in the placebo group, p= 0.012) and LV mass indexed to BSA (−1.32 ± 2.84 g/m^{2}vs. +0.65 ± 3.07 g/m^2 in the placebo group, p = 0.017) in patients with type II diabetes mellitus (311). Their group had previously demonstrated that Allopurinol caused regression of LVM in patients with chronic kidney disease and optimally treated ischaemic heart disease (312) (313)

1.4.2.3 Non-Pharmacological Ways to Regress Left Ventricular Hypertrophy

Weight loss and dietary sodium restriction have long been known to be effective in regressing LVH (172, 314). Syed et al investigated the impact on LVM with weight loss in obese patients (315). Serial echoes were performed on 62 patients post bariatric surgery and after six months or 10% weight loss. Weight loss after bariatric surgery was associated with significant reductions in BP and LVM regardless of other co-morbidities.

The Treatment of Mild Hypertension Study (TOMHS) compared the effects of both pharmacological and non-pharmacological approaches with the treatment of hypertension on LVH regression. It included 844 mildly hypertensive patients in a double-blind, placebo-controlled trial. The patients were advised weight loss and dietary salt reduction, nutritional-hygienic intervention (NH), or NH intervention and randomised to one of the five classes of antihypertensives: including a diuretic (chlorthalidone), beta blocker (acebutolol), alpha1-antagnosit (doxazosin), calcium channel antagonist (amlodipine) and an angiotensin-converting enzyme inhibitor (enalapril). Serial echoes were performed over four years. All groups showed significant decreases in LVM from baseline at three months and continued for 48 months. Interestingly, there was a smaller decrease in BP in the NH intervention only arm (9 vs. 13.3 mmHg). Changes in weight, urinary sodium excretion and systolic BP were moderately correlated with changes in LVM.

The effects of LVH of a non-pharmacological treatment program based mainly on sodium restriction was investigated by Jula et al (316). Serial echoes were performed on 76 previously untreated participants with uncomplicated mild to moderate hypertension. After 12 months of sodium restriction, LVM decreased by 5.4% and LVMI decreased by 4.7% whereas no changes occurred in these parameters in the control group.

1.4.2.4 Summary

In summary controlling BP and using drugs that block the RAS are the standard approach to the management of LVH but this approach is only partially effective since 44% of all patients with T2D are normotensive with LVH. Therefore normotensive LVH is very common. Indeed, BP only contributes 25% to the variability in LV mass seen in a population. Despite a "normal" BP, normotensive LVH is just as risky as is hypertensive LVH. Nevertheless, regressing LVH irrespective of BP changes is an effective way to reduce the incidence of all major CV events as proved conclusively by the LIFE trial. Since controlling BP and using an ACEi or ARB is only partially effective at regressing LVH other ways should be considered. These include weight loss, tight glycaemic control (reduced insulin resistance) and dietary sodium restriction.

Sodium-Glucose-Co-Transporter 2 (SGLT2) inhibitors have been shown to reduce BP, promote sodium natriuresis, improve glycaemic control and cause weight loss. In summary, SGLT 2 inhibitors may improve cardiac function because they appear to reduce the four main causes of LVH; glycaemia/insulin resistance, weight, pre-load and afterload (BP). Since LVH regression is effective regardless of BP we thought it would be valuable to study the SGLT 2 inhibitor dapagliflozin as a potential way of decreasing LVM in diabetic patients with LVH and normal BP.

1.5 Sodium-Glucose Cotransporter 2 Inhibitors and Dapagliflozin

1.5.1 History of the Sodium-Glucose Cotransporter 2 Inhibitor

Dapagliflozin is a SGLT2 Inhibitor which was first approved in Europe in 2012, and the United States Food and Drug Administration (FDA) committee approved it for the treatment of type 2 diabetes in January 2014 (317).

Renal glucose reabsorption kinetics were first demonstrated in the 1930s and the two main transporters in the proximal tubule were characterised as SGLT 1 (high affinity, low capacity) and SGLT2 (low affinity, high capacity) (317, 318). Phlorizin, a glucoside isolated from the bark of apple trees in 1835 is known to be the first natural product with SGLT inhibitory activity Figure 15 (317, 319). Around half a century later it was discovered that Phlorizin inhibited renal glucose

resorption and increased glucose urinary excretion (319). Numerous in vivo studies using diabetic animal models showed that Phlorizin decreased blood glucose concentrations and increased insulin sensitivity (320-322). Katsuno et al subsequently showed that phlorizin inhibited both SGLT1 and SGLT2 (323)

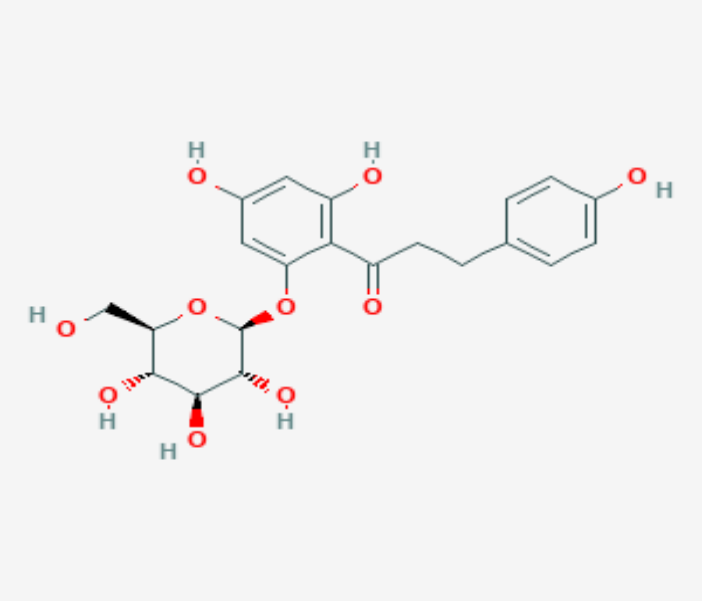


Figure 15 Phlorizin – A gluoside of phloretin attached to a beta-D-glucophyranosyl residue (317)

The use of Phlorizin, as a treatment for diabetes was however limited due to a number of drawbacks. Firstly, due to its non-selective inhibition of both SGLT1 and SGLT2, the inhibition of SGLT1, which is primarily localised in the small intestine led to a number of undesirable gastrointestinal side effects (324). Secondly, Phlorizin is readily metabolised to Phloretin by the hydrolytic enzyme glucosidase in the small intestine and therefore is poorly absorbed and due to this low oral bioavailability must be given parenterally (325). Thirdly, Phloretin is a potent inhibitor of the Glucose transporter 1 (GLUT1) the suppression of which obstructs glucose uptake in various tissues including the central nervous system (326).

Consequently, pharmaceutical research pursued phlorizin derivatives that possessed increased stability/bioavailability and SGLT2 selectivity. Accordingly, SGLT2 inhibitors were developed

initially using O-glucoside analogues. This consisted of having the glucoside moiety linked via an O-linkage to the distal phenolic ring/functional group. The first developed was T-1095 (325).

However, these analogues remained susceptible to B-glucosidases and the incomplete selectivity for SGLT2 led to their discontinuation in early development (325).
Therefore, focus turned towards other derivatives of Phlorizin, C-glucosides whereby the glucoside moiety is linked via a C-linkage (glycosidic oxygen is replaced by carbon) to the phenolic ring. These glycosidic bonds are far more resistant to hydrolysis. Consequently in 2008, Meng et al developed Dapagliflozin (327). Since the development of dapagliflozin several C-glucoside inhibitors have been subsequently developed including empagliflozin and canagliflozin.

1.5.2 Sodium-Glucose Cotransporter 2 Inhibitor

1.5.2.1 Structure

Dapagliflozin is a C-linked glucoside comprising of beta-D-glucose in which the hydroxyl group is replaced by by a 4-chloro-3-(4-ethoxybenzyl)phenyl group Figure 16(324).

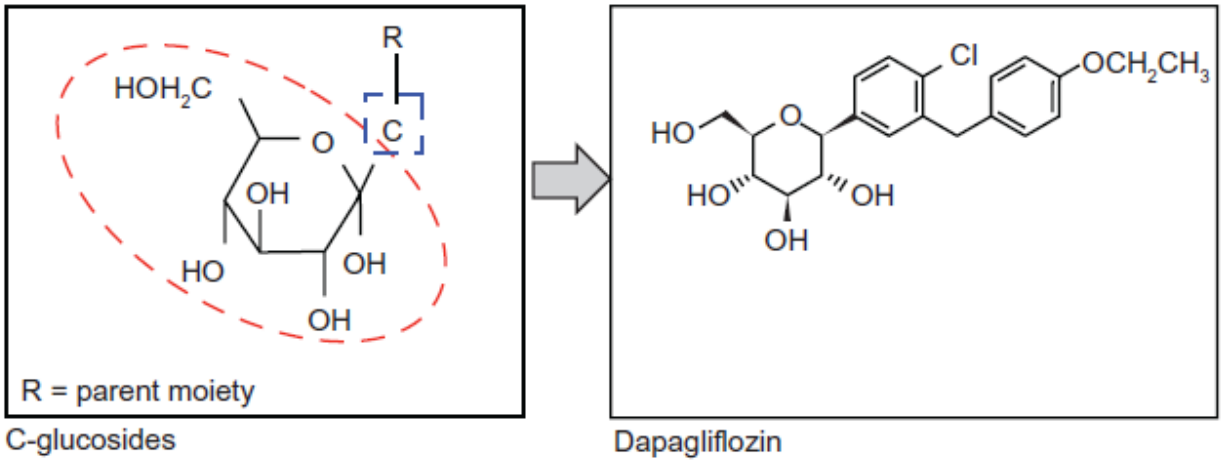


Figure 16 Structural formula of Dapagliflozin which is a C-glucoside analogue (324)

The attachment of the functional group to the glucose by carbon-carbon bond as shown in figure 1.5.2 above results in metabolic stability against glucosidase enzymes.
Its molecular weight is 408.87g/mol. It is described chemically as (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol and its molecular formula is $C_{21}H_{25}CI0_6$.

1.5.2.2 Clinical Pharmacokinetics

Pharmacokinetic data acquired from numerous studies using dapagliflozin doses of 0.1-500mg in healthy subjects with normal renal and hepatic function are summarised below;

In humans, dapagliflozin is quickly absorbed from the gastrointestinal tract with an oral bioavailability of 78% (328). The systemic exposures of dapagliflozin increase in a dose-dependent manner for doses ranging from 0.1-500mg. The half-life of dapagliflozin at the clinical dose of 10mg is 12.5 hours and the inhibition of urinary glucose reabsorption is sustained over 24 hours making it suitable for once-daily dosing (329). Maximal peak plasma concentrations are achieved after around 1-2 hours from administration when fasted. With food, the time to peak plasma concentration is increased by about 1 hour with around 30-45% reduction in the peak plasma drug concentration (330). The overall dapagliflozin systemic exposure is however unaffected and the lower concentrations of dapagliflozin with food still provide maximal glucosuria (330)

The average volume of distribution for dapagliflozin following intravenous administration is 118L. This is greater than the predicted plasma volume and therefore suggests significant extravascular tissue distribution (328).

Dapagliflozin is extensively metabolised following administration although no metabolites with significant pharmacological activity or toxological concerns are formed.
Dapagliflozin is primarily metabolised by uridine diphosphate glucuronosyltransferase (UGT) 1A9-dependent glucuronide conjugation to dapagliflozin 3-O-glucuronide (D30G), which is 2600-

fold less potent than the parent drug with regards to SGLT2 inhibition (331). In vitro data confirms that D30G is formed in both the kidney and the liver (332, 333). In healthy subjects given a single 50mg of (14C) dapagliflozin, D30G was the major drug related component in the plasma (42% of the total drug substance) while the parent drug was 39%. No other metabolite detected in human plasma constituted >5% of total drug substance. 61% of the administered dose of dapagliflozin was recovered in the urine as D30G. Less than 2% of the administered dose of dapagliflozin is excreted in the urine was unchanged dapagliflozin (334).

To evaluate the effects of renal impairment on the pharmacokinetics and pharmacodynamics of renal impairment, a single 50mg dose of dapagliflozin was given to healthy subjects and those with T2D with either normal kidney function or mild, moderate and severe renal dysfunction (38 in total) (333). In those with renal impairment the plasma concentration of dapagliflozin and D3OG incrementally increased with worsening renal function. The efficacy of dapagliflozin was attenuated in renally impaired subjects. This is because with declining renal function as the glomerular filtration rate (GFR) decreases, less glucose is delivered to the proximal renal tubule at the same concentrations of plasma glucose. Therefore, with less glucose in the tubule to be reabsorbed the efficacy of dapagliflozin is reduced. Indeed the steady-state renal glucose clearance was reduced by 42, 83, and 84% in patients with mild, moderate or severe renal impairment respectively (333). It for this reason dapagliflozin is currently not recommended for patients with a GFR of less than 60ml/min/1.73m^2 (335).

With regards hepatic function this was evaluated in a study involving 24 participants with mild, moderate or severe hepatic impairment (Child-Pugh classification). Following a single 10mg oral dose systemic exposure to dapagliflozin in subjects with hepatic impairment correlated with the degree of hepatic impairment (332). The single 10mg doses were generally well tolerated and therefore there is no dose adjustment recommended for patients with mild to moderate hepatic impairment (335). For those with severe hepatic impairment a lower starting dose of 5mg is recommended (335).

1.5.2.3 Clinical Pharmacodynamics

As discussed above dapagliflozin is an SGLT2 inhibitor. In healthy adults, the renal proximal tubule reabsorbs all the filtered glucose (~180g/day). Renal glucose reabsorption requires

basolateral removal of sodium by the active sodium potassium pump. This provides the electrochemical gradient for apical glucose entry via Na^+ -driven sodium-glucose cotransport. Glucose exits the cells into the bloodstream on the basolateral side by following its concentration gradient primarily via GLUT2. Under normoglycaemic conditions ~97% of the filtered glucose is reabsorbed by SGLT2 present in the luminal membrane of the early segments of the proximal tubule. In contrast, SGLT1 expressed in the luminal membrane of the latter segments of the proximal tubule reabsorbs the remaining ~3%. SGLT1 can increase its capacity for glucose reabsorption during hyperglycaemia or SGLT2 inhibition due to the enhanced delivery of glucose to the late proximal tubule as shown in Figure 17.

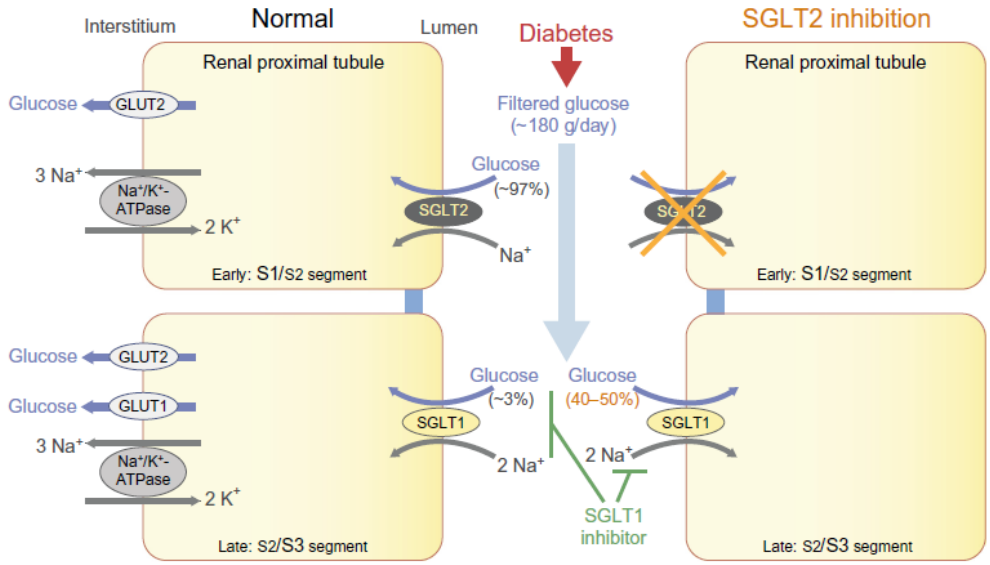


Figure 17 Glucose handling in the proximal renal tubule of the kidney and the effect of SGLT2 inhibition (336)

Dapagliflozin is a potent, competitive, reversible, highly selective and orally active inhibitor of SGLT2 (327). Dapagliflozin demonstrated more than 1200-fold higher affinity for human SGLT2 than SGLT1(327). As a consequence, dapagliflozin results in a dose dependant increase in urinary glucose excretion accompanied by an osmotic diuresis measured as increased urinary flow. The efficacy of dapagliflozin has been evaluated in a number of studies and it has been shown to decrease plasma glucose concentrations and HbA1c (337-339).

Dapagliflozin in general is well tolerated. The incidence of adverse events in clinical trials of SGLT2 inhibitors is similar to that observed with other anti-diabetic medications. The overall

incidence of adverse events ranges from 57.3% to 83.0% while serious adverse events vary between 1.0% and 12.6% (340).

The most frequently noticed adverse events are uncomplicated urinary tract infections and genital infections especially vulvo-vaginitis in women and balanitis in uncircumscribed men (341). This is due to the increased glucose load in the urinary tract which encourages fungal and bacterial growth.
Whilst relatively frequent, most of these infections are generally mild. However, cases of life threatening urosepsis from pyelonephritis in patients on SGLT2 inhibitors have been reported to the FDA (342). Recently the FDA also issued a warning that SGLT2 inhibitors may be associated with a rare but serious infection called necrotizing fasciitis of the perineum otherwise known as Fournier's gangrene. During the five years from March 2013 to May 2018, 12 cases of Fournier's gangrene had been reported in patients taking an SGLT2 inhibitor (343).

The non-insulin based mechanism of action means that the risk of hypoglycaemia is low. This is partly due to the compensatory increase in glucose reabsorption by SGLT1 in the latter proximal tubule segments in addition to the initiation of metabolic counter regulatory mechanisms such as hepatic gluconeogenesis (341). Hypoglycaemia may be an issue though when SGLT2 inhibitors are used in conjunction with other anti-diabetic medication such as sulphonylureas and insulin and dose reductions of these agents should be considered to avoid this.

Ketoacidosis is one of the most serious and potentially life-threatening complication of diabetes. It occurs as a consequence of absolute or relative insulin deficiency and therefore it occurs most frequently in patients with type 1 diabetes. However, in May 2015 the FDA issued a warning that SGLT2 inhibitors may result in euglycaemic ketoacidosis after 73 cases were reported between March 2013 and May 2015 (342). As discussed, SGLT2 inhibitors lower blood glucose concentrations by increasing urinary glucose excretion. This reduces insulin secretion from pancreatic β-cells. The resulting relative insulin deficiency and increase in endogenous glucagon, shift metabolism to more lipolysis and ketogenesis resulting in potential ketosis. As, SGLT2 inhibitors potentially render the body susceptible to ketogenesis whilst continuing to produce glycosuria by lowering the renal glucose excretion threshold, glucose concentrations may be less

abnormally elevated than usually seen in diabetic ketoacidosis (344). Euglycaemic diabetic ketoacidosis seen with SGLT2 inhibitors is usually exacerbated by low carbohydrate intake, insulin omission or dose reductions, dehydration, concurrent illness and surgical stress states (345). If such situations develop temporary cessation of the SGLT2 inhibitor is advised to limit the risk of ketoacidosis.
Interestingly modest ketosis maybe one of the reasons for the potential health benefits of dapagliflozin and other SGLT2 inhibitors and therefore will be discussed in more detail in the next section.

Due to the osmotic diuresis associated with reduce renal tubular glucose absorption increased urinary frequency is often reported. The extra diuresis on average is around 400mls (one extra void per day) (346). This does have the potential to cause volume depletion, dehydration and orthostatic hypotension. This is primarily the reason for caution when using dapagliflozin in the elderly (over 75 years old) and in those on concomitant loop diuretics such as furosemide (335). Despite this theoretical risk, in randomised controlled trials these adverse events are uncommon (347). The diuresis seen with SGLT2 inhibitors such as dapagliflozin also explains the slight transient increase in serum creatinine and blood urea (341).

Other side effects listed by the BNF (76 September 2018-March 2019) include, constipation (likely related to dehydration), dyslipidaemia, sweating and less commonly, nausea and rash (335).

Dapagliflozin does not appear to exhibit any clinically significant drug to drug interactions although caution should be used with concomitant prescription of loop diuretics as discussed above. Importantly dapagliflozin can be combined with all other antidiabetic drugs including metformin, sulphonylureas, gliptins, GLP-1 agonists and insulin. Dose reductions of sulphonlyureas and Insulin should however be considered to limit the risk of hypoglycaemia. Whilst the use of glitazones with SGLT2 inhibitors like dapagliflozin is not currently licensed there is no known interaction between these medications. The clinical trial data where dapagliflozin has been added to pioglitazone is favourable with reductions in $HbA1_C$ and body weight noted without significant increase in the rates of hypoglycaemia (337). Indeed, the

combination of glitazones and SGLT2 inhibitors, is not uncommon in clinical practice in the real world.

1.6 SGLT2 Inhibitors and Cardiovascular Protection

1.6.1 Introduction

In November 2018 results of the Phase III DECLARE-TIMI 58 cardiovascular outcomes trial was presented at the American Heart Association (AHA). Dapagliflozin achieved a statistically significant reduction in the composite endpoint of hospitalisation for heart failure or CV death (348). Additionally, fewer major adverse cardiac events (MACE) were observed. This follows on from the two previously published large CV outcome trials demonstrating similar CV benefits with the SGLT2 inhibitors empagliflozin (EMPA-REG OUTCOME) and canagliflozin (CANVAS) (349, 350)In the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients Removing Excess Glucose (EMPA-REG OUTCOME) study, over 7,000 participants with T2D with coronary, peripheral or cerebrovascular disease were randomised to receive empagliflozin or placebo (349). Empagliflozin was shown to reduce all the three primary major adverse cardiac events (CV death, non-fatal MI and non-fatal stroke). However, what was far more striking was the profound early effects of empagliflozin on CV death and hospitalisation for heart failure, which were reduced by 38% and 35%, respectively. All cause-mortality was reduced by 32%. Importantly, the rates of MI and stroke remained unchanged with empagliflozin therapy. This means that the reductions in CV death are not accounted for reductions in atherothrombotic outcomes. Heart failure was also the most sensitive outcome in the Canagliflozin Cardiovascular Assessment Study (CANVAS) Program. Over 10,000 participants with T2D with either multiple CV risk factors or established CVD were randomised to canagliflozin or placebo (350). The trial produced almost identical reductions in the rates of hospitalisation for heart failure (HR 0.67 in the CANVAS Program and HR 0.65 in the EMPA-REG OUTCOME study). This reminds us of the burden of heart failure in diabetes and that the adverse CV outcomes in patients with diabetes is not simply due to increased atherothrombotic events. The repeated positive results seen in these three large outcome trials also suggest that the result seen in EMPA-REG OUTCOME were not specific to empagliflozin, rather a class effect of SGLT2 inhibitors.

Following on from these three large CV outcome trials most recently the Dapa-HF trial has reported positive results showing that dapagliflozin statistically reduced cardiovascular death or the worsening of heart failure (351).
Therefore there is interest in the potential mechanisms underlying SGLT2 inhibitor associated CV benefits. Some of the key mechanistic themes that have emerged to explain the CV benefits of SGLT2 inhibitors will be discussed below including the potential mechanisms by which SGLT2 inhibitors may regress LVH.

Proposed mechanisms for the CV benefits and potential ways by which dapagliflozin and other SGLT2 inhibitors may regress LVH can be separated into systemic effects (by haemodynamic actions via natriuresis or metabolic actions via glycosuria) and into direct cardiac mechanisms.

1.6.2 Systemic Effects

1.6.2.1 SGLT2 Inhibitors and blood pressure control

Clinical trials have consistently shown that SGLT2-inhibitors lead to a reduction in systolic BP in the range of 3-5mmHg and around 2-3mmHg in diastolic BP in patients with T2D (352). In addition, others have explored the effect of SGLT2 inhibition on circadian B rhythms. The restoration and maintenance of circadian rhythm is imperative to CV health (353). Loss of nocturnal decline in BP has been established as an important marker for CV risk, independent of overall BP during a 24 hour period (354). Animal studies with obese salt sensitive rats have shown that SGLT2 inhibition may restore normal nocturnal dipping(355). Recently, a clinical case study examined dapagliflozin in patients with T2D who exhibited non-dipper type BP defined as a sleep-time mean SBP greater than 90% of awake-time mean) (356). Dapagliflozin significantly reduced BP and altered the circadian dipping pattern of BP, from a non-dipper type to a dipper type i.e. sleep mean SBP <90% of awake-time mean. Other trials using empagliflozin and canagliflozin have shown they successfully reduce systolic BP at night (357, 358).
The BP reduction effect of SGLT2 inhibitors occurs without a compensatory increase in heart rate, suggesting a lack of compensatory sympathetic activation (359, 360). This is an important observation as chronic elevation of sympathetic nervous activity (SNA) not only causes

hypertension, but increases arterial stiffness, causes endothelial dysfunction and alters renal sodium and water homeostasis to promote fluid retention and oedema (361-365). SNA as discussed earlier also enhances cardiac hypertrophy via β-adrenergic signalling (366).

SNA therefore strongly correlates with CV mortality and is associated with a poor prognosis in patients with HF (367, 368). In keeping with the hypothesis that SGLT2 inhibitors suppress SNA trials have shown them to improve the circadian rhythm of sympathetic activity in rats with metabolic syndrome (369). Matthews et al demonstrated that SNA is upregulated in obesity and T2D and that dapagliflozin reduces SNA markers, such as tyrosine hydroxylase and noradrenaline (370).

There are several factors involved to explain the likely mechanisms by which SGLT2 inhibitors lower BP. The first of which is a reduction in plasma volume. SGLT 2 inhibitors cause an acute contraction in plasma volume within hours of initiation, which accounts for a 3-7% increase in haemoglobin, albumin and urea concentrations (371). Indeed, within one week of treatment with SGLT2 inhibitors, body weight drops significantly which correlates better with sodium and water loss than with calorific loss due to glycosuria (372). Recent studies have also demonstrated important differences between SGLT2 inhibitors and classical diuretics. In a comparative study of hydrochorothiazide and a dapagliflozin, a persistent 7% reduction in plasma volume and an increase in erythrocyte mass was noted with dapagliflozin but not with hydrochorothiazine over a 12 week period of therapy (373).

The underlying mechanism for BP reduction with SGLT2 inhibition is not merely natriuresis however, as the natriuresis with SGLT2 inhibition is modest when compared to conventional diuretics (374). Nor it is dependent on the osmotic diuretic effects of glycosuria as the BP lowering effect is observed even in patients without diabetes and in those with a reduced GFR despite minimal glycosuria (375) (376).

A second mechanism that may play some part in the BP-lowering effect observed with SGLT 2 inhibitors is a reduction in arterial stiffness. Arterial stiffness is associated with hypertension, obesity and hyperglycaemia (377, 378). It is known to be a predictor of CV events, heart failure and death especially in patients with diabetes (379-382). SGLT 2 inhibitors have been shown to reduce arterial stiffness, demonstrated by pulse wave velocity and augmentation index in young patients with T1D (359). Markers of arterial stiffness such as pulse pressure have also been shown

to improve in patients with T2D with SGLT2 inhibition (383). The underlying mechanisms for this observation are not clearly known although reduction of total body sodium might be one of the mechanisms. Sodium overload is associated with damage to the endothelial glycocalyx in HF, resulting in decreased bio-availability of NO for vascular smooth muscle relaxation (384).

The activity of RAAS during studies with SGLT2 inhibitors has been found to be slightly increased but within normal parameters and is likely to be a compensatory response to reductions in intravascular volume and BP (385, 386). Based on this data it is unlikely that suppression of RAAS contributes to the antihypertensive effect of SGLT2 inhibitors. However, urinary ACE2 protein and enzymatic activity appears to increase (386). This is potentially important for BP lowering, as the ACE2-Ang(1-7) pathway promotes vasodilatation.

In summary natriuresis and the reduction of arterial stiffness are the most significant mediators responsible for the antihypertensive effects of SGLT2 inhibitors. Changes in neurohormones do not seem to account for the BP-lowering effects with the possible exception of ACE2-(Ang1-7) pathways although the absence of SNA is also important.

Whilst BP reduction given its close association with LVH is likely to be a significant mechanism for the potential for dapagliflozin to regress LVH, the BP reduction seen with SGLT2 inhibition does not fully account for the CV benefits seen in the large CV outcome trials.

It is unlikely that a 4mmHg reduction in systolic BP can fully account for the substantial benefits given the modest impact of BP reduction on HF or mortality. Furthermore, if the antihypertensive effect did account for the improved outcomes one might have expected to see more benefit in the plaque-rupture atherothrombotic events such as non-fatal stroke or MI.

1.6.2.2 SGLT2 Inhibitors and Their Diuretic Effect

As touched upon in the mechanisms of BP reduction with SGLT2 inhibition they can reduce plasma volume secondary to natriuresis and osmotic diuresis from glycosuria. Individuals with diabetes are known to have an increase in whole body sodium and water content. Dapagliflozin has been shown to reduce tissue sodium content in patients with T2D (387). As a consequence, SGLT inhibitors could induce relative euvolaemia. This plasma volume reduction reduces preload and could easily explain the reduction in HHF seen with SGLT2 therapy. Reduction in preload

could also reduce myocardial stretch with could reduce cardiac arrhythmogenesis. Indeed, mediation analysis from the EMPAREG OUTCOME trial has suggested that volume contraction is likely a key component of the CV benefit noted in the trial. It has been suggested that approximately 50% of the CV benefit seen in the trial could be attributed to empagliflozin induced haemoconcentration (388). An early haemodynamic benefit would also fit with the early separation of the Kaplan-Meier curves noted in the big SGLT2 outcome trials when comparing SGLT2 inhibitors with placebo. Reduction in preload will improve the ventricular loading conditions reducing LV wall stress and could contribute to the potential for an SGLT2 inhibitor such as dapagliflozin to regress LVM in patients with LVH.

However, can diuresis really explain the CV benefits with SGLT 2 inhibition treatment? The most commonly used diuretics produce similar or greater reductions in intravascular volume and net sodium balance but have not changed prognosis in heart failure.

There is however, important differences between SGLT2 inhibitors and classical diuretics. As alluded to earlier Lambers Heerspink et al showed that SGLT2 inhibitors appear to produce a sustained reduction in plasma volume when compared with other diuretics such as the thiazide hydrochlorothiazide (373). In addition they demonstrated a rise in haematocrit with Dapagliflozin that was not seen with hydrochlorothiazide. A decrease in plasma volume, with resultant haemoconcentration could contribute to this observed increase in haematocrit. However, an alternative explanation is that Dapagliflozin may have a direct effect on red cell mass as suggested by the transient increases in reticulocyte count and serum erythropoietin concentrations also seen in the study. One theory for this is that when glucose reabsorption in the proximal tubules is inhibited by SGLT2 inhibitors, oxygen consumption by the cells of the proximal tubules decreases (389). This may lead to improvement of local hypoxia in the region around the proximal tubules of diabetic patients and increase the capacity for erythropoietin synthesis by stromal fibroblasts in the proximal tubules. The elevated haematocrit may therefore be a surrogate marker for the recovery of tubulointerstitial function. Indeed some have therefore suggested that SGLT2 inhibitors can be thought of as “beta-blockers for the kidney” because these drugs help to reverse renal remodelling by reducing the workload that diabetes imposes on the tubulointerstitial tissues (390).

If has been proposed that this increased haematocrit may result in better oxygen transport and therefore contribute to improved cardiac function and outcomes particularly when combined with the metabolic benefits to be discussed later (391).

Another study compared dapagliflozin with the loop diuretic bumetanide (392). Both drugs reduced body sodium and interstitial fluid but dapagliflozin did this with little or no change in blood volume whereas bumetanide produced far greater reductions in intravascular volume. This differential effect in regulating interstitial fluid rather than intravascular volume may be important in patients with heart failure as many are actually intravascularly deplete. This can be aggravated by classical diuretics and the ability of SGLT2 inhibitors to selectively reduce interstitial fluid may limit the reflex neurohormonal stimulation that occurs with intravascular volume contraction. This may also explain the neutral effects on the SNS repeatedly reported with SGLT inhibition. Perhaps the normalisation of plasma volume is insufficient to activate the SNS.

In addition to this SGLT2 inhibitors are unique among diuretics available in that they exert their effect in the proximal tubule of the kidney. SGLT 2 inhibition therefore results in an increased delivery of sodium and chloride to the macula densa in the loop of henle downstream which may also limit the activation of the RAAS and SNS both of which can have an adverse effect of CV remodelling and subsequent CV outcomes.

The blockade of proximal sodium chloride absorption results in increased uptake of sodium chloride via the Na/K/2Cl transporter in the loop of henle. This is an energy requiring process leading to the breakdown of adenosine triphosphate (ATP) to adenosine which then acts via adenosine 1 receptors on afferent arteriolar vascular smooth muscle causing vasoconstriction (393). This reduces intraglomerular hypertension and clinically these effects are manifested by reductions in acute reductions in albuminuria and GFR (394). This may also explain why long-term treatment with SGLT2 inhibitors is associated with reduced progression of albuminuria and slower decline in renal function when compared with placebo (395). Albuminuria has long been established as an independent CV risk predictor. Indeed it may be that the CV benefits observed with SGLT2 inhibition may be secondary to the preservation of renal function. The maintenance of total body salt and water homeostasis without the activation of the SNS and the inflammation associated with diabetic nephropathy may help reduce adverse LV remodelling and be responsible for reducing heart failure as summarised in Figure 18.

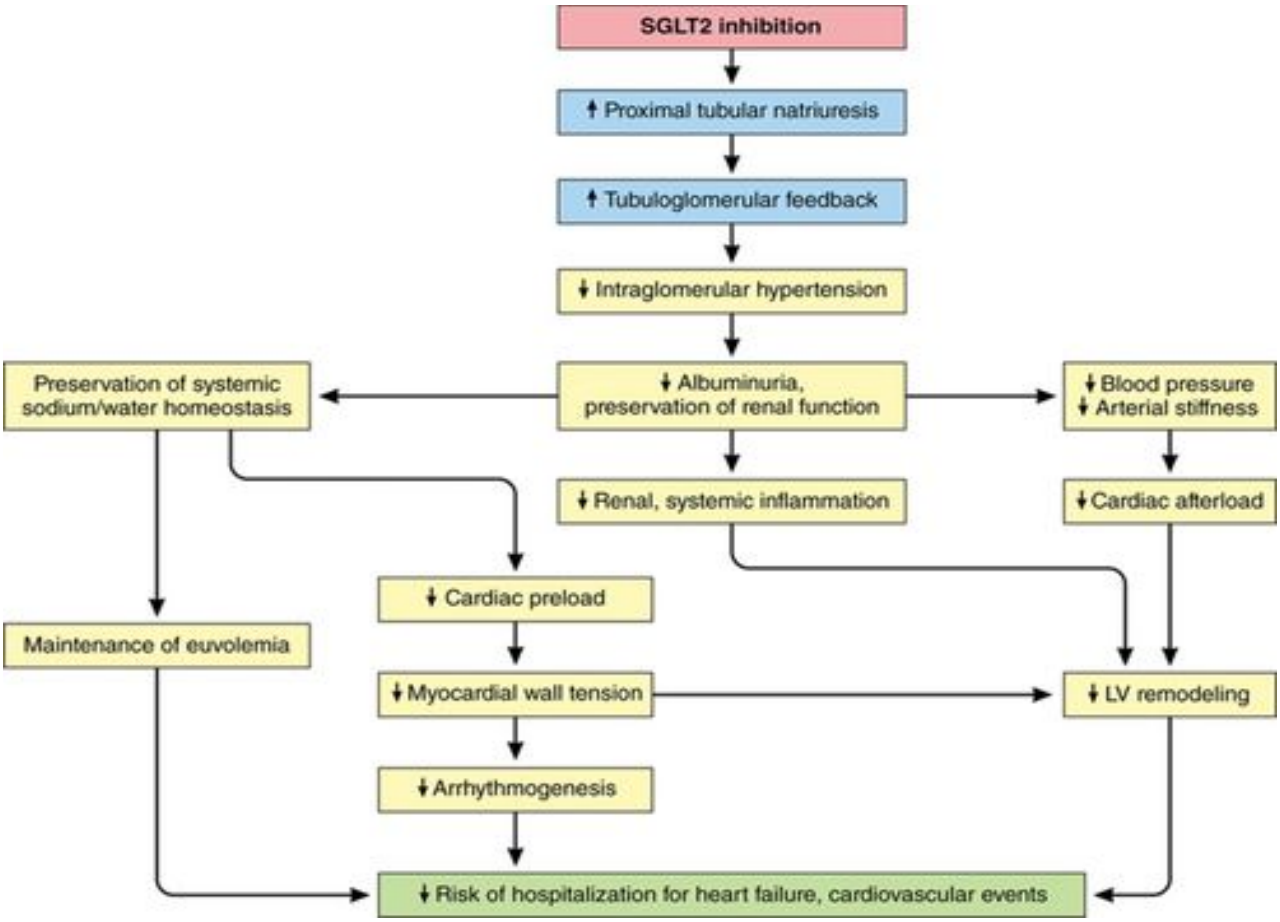


Figure 18 The Renal-Cardio hypothesis for cardiovascular protection with SGLT2 Inhibition (396)

Finally, SGLT2 inhibitors reduce serum uric acid concentrations unlike loop and thiazide diuretics which both raise them (397). Higher serum uric acid concentrations are associated with CVD and therefore the SGLT2 associated uricosuria and uric acid lowering may be beneficial although the clinical relevance of this remains uncertain.

So, it is clear that SGLT2 inhibitors such as dapagliflozin have potential to improve ventricular loading conditions through both a reduction in preload (secondary to natriuresis, osmotic diuresis) and afterload (reduction in BP and improvement in arterial stiffness). They also have a number of metabolic actions which will be discussed below.

1.6.2.3 SGLT2 Inhibitors and Their Effect on Body Fat and Fat Mass

SGLT2 inhibitors have consistently been shown to lead to weight reduction of 2-3kg that occurs gradually over the first few months on treatment. This is a consistent finding across the class of medications (352). Initially fluid loss will contribute to this following osmotic diuresis (372). The glycosuria induced by these agents is typically associated with a net calorie loss of approximately 200-300 kilocalories per day (398). The weight loss however does appear to plateau after 3-6 months (399). With SGLT2 inhibition, even when circulating glucose concentrations are reduced, whilst glycosuria does decrease it remains elevated even in those who achieve near-normal glucose control (400, 401). The reason for the lesser weight loss is therefore not entirely clear but it is believed it may be due to compensatory increased energy intake as SGLT2 inhibitors have no effect on energy expenditure (399, 402).

Of greater interest when thinking of the potential CV benefits of SGLT inhibition is that studies have demonstrated that the weight loss during SGLT2 inhibitor treatment was due to total fat mass reduction including visceral adiposity. Visceral fat is well recognised to be associated with an increased risk of T2D, CV complications and overall mortality. In dedicated body composition studies comparing canagliflozin with glimepiride for 52 weeks, dapagliflozin with placebo for 104 weeks and empagliflozin with glimepiride over 104 weeks and assessing visceral adiposity mass by dual-energy X-ray absorptiometry, computed tomography imaging or magnetic resonance imaging, it was demonstrated that the majority of weight loss with SGLT2 inhibition was due to a reduction in visceral or subcutaneous fat (403-405). Even in shorter studies investigating the changes in indirect markers of visceral adiposity, that is, visceral adiposity index and waist circumference, significant reductions have been demonstrated (406-408).

Altered adipokine production has been suggested as a common mechanism in obese subjects through which CVD and insulin resistance develops (409). Some studies have suggested that SGLT2 inhibitors mediate their benefit by resetting the balance between pro- and anti-inflammatory adipokines. As discussed earlier leptin is thought to be detrimental to CV health due to its role in sodium regulation as well as cardiac inflammation and fibrosis. Whilst data on the effect on circulating adipokines are currently sparse, canagliflozin has been shown to reduce serum leptin by 25% and increase the concentrations of the anti-inflammatory adipokine adiponectin by 17% when compared to glimepiride (410). This was associated with a marked reduction in the

inflammatory cytokine IL-6. Dapagliflozin has also been shown to significantly increase adiponectin concentrations when compared with other non SGLT2 oral hypoglycaemic treatment (411).

The BP reducing effect of SGLT2 inhibition will also have been contributed to by the weight loss effect seen with SGLT 2 treatment. Metabolic effects associated with SGLT2 inhibitors which will be discussed in more detail in the next section including increased lipolysis, fat oxidation, ketogenesis and decreased insulin secretion plus increased glucagon release also contribute to the loss of fat and body weight (412).

SGLT 2 inhibitors therefore in summary clearly have the potential to modify both the direct and indirect adverse effects of obesity on the myocardium described earlier which contribute to the structural changes seen in diabetic cardiomyopathy including LVH.

1.6.2.4 SGLT2 Inhibitors and Glycaemic Control and Insulin Resistance

Meta-analyses for dapagliflozin, empagliflozin and canagliflozin suggest they lower HbA1c concentrations between 0.7 and 0.8% relative to placebo (413). To date however there is little proof that improved glycaemic control affects the risk of CV events. The three largest trials ADVANCE, ACCORD and VADT which enrolled patients with longstanding T2D failed to show a significant reduction in macrovascular CV events with more intensive glucose-lowering strategies when compared to standard care (414-416).

SGLT2 inhibitors are however different to other glucose-lowering strategies since they remove glucose from the body rather than stimulating tissue glucose uptake which occurs with insulin, incretins, and sulphonylureas. This is an important point to make as whilst these other hypoglycaemic agents may lower blood glucose concentrations , glucose influx into cells remains elevated. In contrast SGLT2 inhibitors result in less glucose influx into cells theoretically limiting cellular glucose toxicity by the metabolic pathways described earlier. Similarly Metformin another

diabetic medication thought to be cardioprotective acts mainly in the intestine and therefore also limits cellular glucose influx and subsequent toxicity (417, 418).

In patients with T2D, empagliflozin 25mg once daily for two weeks was shown to improve β-cell function, measured as insulin secretion/insulin resistance index (419). Another metabolic study in patients with T2D confirmed that glucose reduction with empagliflozin reduced glucose toxicity and improved pancreatic β-cell function (402). It also confirmed that insulin sensitivity of tissue glucose uptake was improved. A glucose clamp study (so-called because blood glucose is held or "clamped" at a certain concentration) also demonstrated that dapagliflozin improves insulin sensitivity in patients with T2D (420). Given the direct link between insulin resistance and accelerated CVD and the increasing understanding of its importance in the pathogenesis of LVH and diabetic cardiomyopathy these findings are potentially highly significant (421).

The loss of glucose into the urine following treatment with SGLT2 inhibitors triggers several compensatory pathways one of which is an early increase in plasma glucagon (422). Glucagon promotes hepatic glucose production by increasing glycogenolysis, gluconeogenesis. The increased glucagon to insulin ratio also promotes ketogenesis and SGLT2 inhibitors are known to increase the production of the ketone body β-hydroxybutyrate (423). The myocardium is the highest consumer of ketone bodies per unit mass and oxidises ketone bodies in proportion to their delivery (424). This is of interest because as we discussed earlier the diabetic heart has an over-reliance on fatty acids and demonstrates metabolic inflexibility which contributes to contractile dysfunction and decreased cardiac efficiency. Ketone bodies are potentially a super fuel, producing ATP more efficiently than glucose or free fatty acids (425). In addition to this β-hydroxybutyrate combustion results in less production of reactive oxygen species compared to free fatty acid oxidation (426). It is therefore proposed that this increased ketone production may not only improve cardiac function in the failing heart, but also increase its mechanical efficiency however it must be stressed that data to support this theory does remain scarce.

In summary, SGLT2 inhibitors as a hypoglycaemic agent maybe beneficial in limiting the adverse LV remodelling in patients with diabetes as they reduce cellular glucose not just plasma glucose concentrations. They therefore have the potential to limit the adverse consequences of tissue

damage resulting from gluco-toxicity. In addition, they may have the potential to improve cardiac metabolism improving myocardial efficiency and function.

So it is clear that SGLT2 inhibitors such as dapagliflozin have many systemic effects on the CV system which have the potential to impact on LVH. However, systemic effects only may not be enough to explain the far superior CV benefits when compared to other glucose-lowering drugs. As discussed earlier the cardiac abnormalities observed in diabetic cardiomyopathy include myocardial hypertrophy, as well as interstitial fibrosis, cardiac apoptosis and necrosis. Evidence is emerging that SGLT2 inhibitors may also have direct effects on the myocardium mediated by their ability to reduce cardiac inflammation, oxidative stress, fibrosis and ionic dyshomeostasis. These effects will be discussed in the next section.

1.6.3 Direct Effects

1.6.3.1 SGLT2 Inhibitors and Their Effect on Cardiac Oxidative Stress

Oxidative stress plays an important role in the development of LVH. SGLT2 inhibitors have been shown to act as antioxidants independently from their glucose lowering effects resulting in reduced cardiac oxidative stress (427). Using a genetic prediabetes/metabolic rat model Kusaka et al demonstrated that 10 weeks empagliflozin significantly reduced superoxide levels in cardiac tissues. Indeed, empagliflozin ameliorated cardiac hypertrophy and fibrosis despite no significant change in BP (428). Similar findings were reported by Lin et al (429). Using mice they also showed that cardiac interstitial fibrosis, cardiac interstitial macrophage infiltration and cardiac superoxide levels were significantly reduced by 10 weeks empagliflozin therapy. Whilst these studies demonstrate the reduction in ROS in diabetic rodents with SGLT2 inhibition, it is difficult to conclude whether this antioxidant effect is directly secondary to the drug themselves or indirectly via decreasing hyperglycaemia and glucotoxicity.

It is important to note at this point that macrophages not only initiate and accentuate inflammation but are also involved in resolution and repair. Macrophages can be referred to as M1 or M2 with M1 macrophages known to be pro-inflammatory and M2 macrophages associated with tissue

repair (430). In contrast to M1 macrophages, M2 macrophages reduce ROS . M2 macrophages also express interleukin IL-10 which is known to inhibit myofibroblast differentiation reducing extracellular matrix production (431). Macrophage phenotyping is regulated by signalling pathways such as the STAT3 signalling pathway which is known to upregulate these protective M2 macrophages. Indeed, antioxidants have previously been shown to increase STAT 3 activity upregulating M2 macrophage activity reducing cardiac inflammation (432).

Using euglycaemic rats Lee et al demonstrated that dapagliflozin directly reduces oxidative stress and cardiac inflammation by mediating M2 macrophage polarisation through the signal transducer and activator of transcription 3 (STAT3) pathway (433). They induced an MI in healthy mice and 24 hours later the mice were randomised to saline or Dapagliflozin for four weeks (433). Post infarction was associated with increased levels of superoxide which was attenuated by SGLT inhibition. SGLT inhibition significantly increased IL-10 and the percentage of M2 macrophage infiltration via increased STAT3 activity and STAT3 nuclear activation. At day 28 after infarction, SGLT inhibition was associated with reduced myofibroblast infiltration and fibrosis. The increased M2 macrophage and myofibroblast infiltration seen with both Dapagliflozin was abolished by the administration of the STAT3 inhibitor S3I-201 confirming the importance of STAT3 in regulating macrophage phenotyping and myocardial myofibroblast infiltration. All together this evidence supports Dapagliflozin as a potential direct antioxidant independent from its glucose lowering effects.

1.6.3.2 SGLT2 Inhibitors and Their Effect on Cardiac Inflammation

Chronic low grade inflammation is increasingly recognised as a key feature associated with T2D and its complications including diabetic cardiomyopathy (434). Unfortunately, no inflammatory markers were measured in the large SGLT2 inhibitor CV outcome trials (348-350). However, recently smaller pilot studies have provided information on the effect of SGLT2 inhibition on serum inflammatory markers. Dapagliflozin as well as empagliflozin and canagliflozin have been shown to reduce serum inflammatory markers such as hsCRP, tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interferon-gamma (IFN-γ) (410, 435-442). It must be stressed though that these reductions were mostly only numerical and failed to reach statistical significance although this may be due to the small number of patients included in these trials. The changes in

serum inflammatory markers and adipokines (discussed earlier) with the three available SGLT2 inhibitors are summarised in Table 6.

References	Gliflozin	Leptin	Adiponectin	hsCRP	TNF-α	IL-6	IFN-γ
Ferrannini et al., 2010	Dapagliflozin	NA	NA	↓	NA	NA	NA
Bailey et al., 2012	Dapagliflozin	↓	↑	NA	NA	NA	NA
Okamoto et al., 2016	Dapagliflozin	NA	↑*	↓*	NA	NA	NA
Matsumura et al., 2017	Canagliflozin	NA	↑*	NA	↓	NA	NA
Hattori, 2017	Empagliflozin	NA	NA	↓	NA	NA	NA
Tobita et al., 2017	Dapagliflozin	NA	↑*	↓	NA	NA	NA
Sato et al., 2018	Dapagliflozin	NA	NA	NA	↓*	NA	NA
Garvey et al., 2018	Canagliflozin	↓	↑	↓	↑	NA	NA
Tan and Tan, 2018	Empagliflozin	NA	NA	NA	↓*	↓*	↓*

These are numerical trends except where * ($P < 0.05$) indicates significant changes.
hsCRP: high-sensitivity C-reactive protein; TNF-α: tumour necrosis factor-alpha; IL-6: interleukin-6; IFN-γ: interferon-gamma; NA: not available;.

Table 6 Changes in serum inflammatory markers with the three most commonly used SGLT2 inhibitors in patients with type 2 diabetes (410, 435-443)

The mechanisms by which SGLT2 inhibitors reduce low grade inflammation are numerous and not mutually exclusive. Many of these are secondary to the systemic effects of SGLT2 inhibition discussed earlier. For example, SGLT2 inhibitors are consistently associated with weight loss and significant reductions in total fat mass including both visceral and subcutaneous adipose tissue (SCAT) (352). Given our knowledge that fat is more than just a simple energy storage compartment and can modulate inflammatory and metabolic processes such fat reduction will contribute to improving low grade inflammation (410).

Studies have shown that acute hyperglycaemia increases cytokines such as IL-6 and TNF-α (444). In mice, canagliflozin prevented post prandial release of the pro-inflammatory cytokine IL-1β in the circulation (445). It is possible that SGLT2 inhibitors reduce the deleterious chronic effects of glucose induced IL-1β release by reducing hyperglycaemia via glycosuria. Hyperinsulinemia in mice has been shown to drive adipose tissue inflammation (446). Antidiabetic medications such as Metformin that reduce circulating insulin concentrations have been associated with reductions of inflammatory markers (447). Due to glycosuria SGLT2 inhibitors reduce circulation insulin concentrations as well as the insulin needs of those requiring exogenous insulin (419). Dapagliflozin has been shown to increase insulin sensitivity and increase insulin clearance further lowering circulating insulin concentrations (448)

Finally, SGLT2 inhibitors are known to reduce ROS and oxidative stress which is closely linked to inflammation. Indeed as discussed above Lee et al demonstrated in Wistar rats with acute MI that Dapagliflozin reduced superoxide levels (433). They also revealed that Dapagliflozin decreased inflammatory cytokines including IL-1β and IL-6 which is likely to be secondary to the increased M2/M1 macrophage ratio.

So it is clear that SGLT2 inhibitors can potentially reduce inflammation via a number of systemic actions. Interestingly, a recent study revealed a possible direct mechanism of SGLT2 inhibitors on cardiac inflammation. Ye et al showed that Dapagliflozin attenuated the activation of the nucleotide binding oligomerization domain-like protein 3 (NLRP-3) (449). NLRP3 is an interleukin -1β family cytokine-activating multi-protein signalling complex (inflammasome) that converts cytokine precursors such as pro-IL-1β into mature biologically active IL-1β via the activation of caspase-1 (427). NLRP3 is known to be upregulated in the heart and be associated with cardiac inflammation in T2D and therefore is implicated in the pathogenesis of diabetic cardiomyopathy (434, 450, 451). Ye et al showed that 8 weeks of treatment with dapaglilflozin in genetic diabetic mice decreased the levels of myocardial MRNA associated with NLRP-3 inflammaosome and pro-inflammatory cytokines such as IL-6, TNFα, caspase-1and IL-β (449). This reduction in NLRP-3 could be argued to be secondary to other systemic effects of SGLT2 inhibition described above. Furthermore, SGLT2 inhibitors are associated with lower circulating concentrations of uric acid (397). Uric acid is a potent activator of the nucleotide binding (NLRP-3) (452)As we have discussed, SGLT2 inhibitors significantly increase ketone bodies such as beta-hydroxybutyrate (BHB) (423). Ketone bodies, in particular BHB can exert anti-inflammatory effects via inhibition of NLRP-3 (453). However, to rule out systemic effects Lee et al also performed in vitro experiments by incubating mouse cardiofibroblasts in a media containing Dapagliflozin for 16 hours (449). Intriguingly, Dapagliflozin still attenuated caspase-1 and IL-1β in a dose dependent manner. Given that SGLT2 do not exist in cardiac tissue these results suggest that the attenuation of NLRP-3 is unrelated from the SGLT2 inhibition and glucose lowering effects of Dapagliflozin.

1.6.3.3 SGLT2 Inhibitors and Their Effect on Cardiac Fibrosis

Cardiac fibrosis is widely regarded as a common final pathway through which heart failure develops. Indeed, nearly all aetiologies of heart disease including LVH involve pathological remodelling characterised by excessive deposition of extracellular matrix proteins by cardiac fibroblasts (454).

In simplistic terms if SGLT2 inhibitors can reduce inflammation and this will clearly have a downstream effect on fibrosis. As discussed above there is experimental data in rat models that Dapagliflozin suppresses collagen synthesis via increasing the polarisation of M2 macrophages with subsequent inhibition of myofibroblast differentiation (433).

However, evidence is also emerging that SGLT2 inhibitors may also have a direct effect on human cardiac fibroblast phenotype and function. Kang et al showed that empagliflozin significantly attenuated TGFβ1 induced fibroblast activation and reduced cell-mediated extracellular matrix remodelling as measured by the collagen fibre alignment index (455). The same series of studies demonstrated that empagliflozin suppressed may key pro-fibrotic markers, including matrix metalloproteinase 2, connective tissue growth factor, α-smooth muscle actin and type 1 collagen. Therefore, SGLT2 inhibition may result in direct and favourable effects on cardiac fibroblast activity independent of hyperglycaemia. Animal models have also demonstrated both empagliflozin and dapagliflozin to reduce cardiac fibrosis (428, 449).

This favourable effect on fibrosis is exciting given the importance of cardiac fibrosis in the pathogenesis of heart failure and LVH.

1.6.3.4 Ionic homeostasis Na^+/H^+ channel

An emerging hypothesis is that SGLT2 inhibitors may directly inhibit the NHE-1 exchanger which it thought to play a key role in cardiac remodelling as discussed earlier (455). Baartscheer and colleagues isolated ventricular myocytes from rabbits and rats and incubated them with empagliflozin for 3hours (456). They demonstrated that empagliflozin lowered cytoplasmic sodium and calcium levels, while increasing mitochondrial calcium levels. These effects were

similar to the effect expected from a NHE-1 inhibitor. This was confirmed in cells pre-treated with the NHE-1 inhibitor cariporide. In these cells empagliflozin had very little effect on cytoplasmic sodium levels.

In addition to this it has recently been demonstrated that both dapagliflozin and canagliflozin also impair NHE-1 activity in mouse myocytes (457). Further evidence for the SGLT2 inhibitor interaction with NHE-1 arises from molecular binding studies. Uthman et al also demonstrated that SGLT2 inhibitors exhibit high binding affinities with the extracellular Na^+ binding site of the NHE-1 (457). All this data supports the theory that SGLT2 inhibitors exert an off-target effect on the NHE-1and therefore have the potential to directly target suspected drivers of cardiac remodelling (i.e. elevated cytosolic Na^+ and Ca^{2+}). This is particularly important in diabetic and failing hearts where the levels of cytosolic Na^+ and Ca^{2+} are known to be elevated (264).

In addition to this it has been demonstrated that empagliflozin increases sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) resulting in an improvement in LV diastolic function (458). SERCA2a is an essential protein regulating Ca^{2+} uptake into the sarcoplasmic reticulum during cardiac muscle relaxation. Jourbert et al confirmed these findings demonstrating that Dapagliflozin increased SERCA2a function in a diabetic lipodystrophic mouse model (459). (460). SERCA2a activity has been shown to decrease in response to pressure overload and diabetes contributing to contributing to cardiac contractile dysfunction and diastolic dysfunction (461, 462). This suggest that improvement in calcium handling through these mechanisms may be responsible for some of the protective effects in heart failure with SGLT2 inhibition.

1.6.3.5 Effect of SGLT2 Inhibitors on Cardiac Structure and Function

In the large CV outcome trials SGLT2 inhibitors primarily reduced CV events through the prevention of heart failure. As discussed at length above SGLT2 inhibitor provide a number of potential systemic and direct benefits which may impact on cardiac structure (LVH) and function. Evidence is beginning to build that SGLT2 inhibitors do indeed improve cardiac structure (LVH regression) and function.

SGLT2 inhibitors have been found to improve cardiac histopathologic changes in diabetic cardiomyopathy models of mice and rats (427). Kusaka et al demonstrated that 10 weeks empagliflozin treatment in genetic prediabetes/metabolic syndrome rats significantly reduced LVweight, cardiomyocyte size, cardiac interstitial fibrosis and cardiac interstitial macrophage infiltration (463). Verma et al in an uncontrolled case series demonstrated that empaglilflozin may reduce LVM (464). Ten patients with T2D and established CV disease were given empagliflozin 10mg/day as per approved clinical indication without any other concurrent changes in medication. Transthoracic echocardiograms were performed before and after 3 months of therapy. This short term empagliflozin therapy was associated with a significant reduction in LVM index (mean (SD) 88 (21) vs 75 (19) g/m^2, p=0.01). Furthermore, empagliflozin therapy appeared to improve diastolic function as assessed by the early lateral annular tissue Doppler velocity (8.5 (1.6) vs 9.6 (1.3) cm/s, p=0.002). The rapid benefits are consistent with the early separation of the Kaplan-Meier curves for heart failure-associated hospitalisation and CV mortality observed in the EMPA-REGOUTCOME trial (349). These findings were however preliminary and only in a small number of patients therefore one cannot draw any definitive conclusions. Very recently the EMPA-HEART trial showed that empagliflozin promotes reverse LV remodelling in patients with diabetes (465). In total 97 patients with T2D with established CAD were randomised to empagliflozin or placebo for 6 months. Patients received a baseline cardiac MRI, with repeat cardiac MRI evaluation at 6 months. Empagliflozin resulted in a significant reduction in LVMI (-2.6 vs -0.01 g/m^2, p =0.01.). There was also a significant reduction in ambulatory BP and an increase in haematocrit. These secondary changes in mean systolic BP and haematocrit suggest empagliflozin favourably affected cardiac preload and afterload which would account for the reduction in LVM. These results were seen in a normotensive diabetic population without heart failure and already on ACE inhibitor or ARB therapy (i.e. on top of excellent standard of care). Given the consistent reduction in hospitalisation with heart failure (HHF) seen in all the three major SGLT2 CV outcome trials this beneficial effect on cardiac remodelling seen with empagliflozin is likely to be a class effect. However, at present studies assessing LVM regression using other SGLT2 inhibitors such as dapagliflozin have not been performed.

The importance of SGLT2 inhibition in diabetic patients with LVH has also been highlighted by a recent subgroup analysis of the EMPA-REG OUTCOME trial which demonstrated that the reduction of CV death, MI and stroke was greater in patients with LVH than in those without

LVH(466). It should be cautioned though that definitive conclusions cannot be made based on this analysis as only a small number of patients were analysed and LVH was defined using ECG which we know to lack sensitivity for detecting LVH. However, it further highlights the interest in whether SGLT2 inhibition causes a regression in LVM.

In addition to beneficial effects on cardiac structure of SGLT2 inhibition evidence is emerging from a number of diabetic cardiomyopathy and myocardial ischaemic models of mice and rats that they also improve cardiac function (427). Eight weeks of treatment with Dapagliflozin in genetic diabetic mice improved EF and fractional shortening (449). Dapagliflozin also improved diastolic function in a diabetic non-obese mouse model (459). Dapagliflozin improved the E/A (early/late diastolic) ratio, isovolumetric relaxation time (IVRT), deceleration time (DT) and end diastolic wall thickness (EDWT).

For empagliflozin, a number of studies have shown it provides more benefits to diastolic function than to systolic function (427). Even if the benefits of SGLT2 inhibition do not extend to LV systolic function in everyday practice the potential improvements in diastolic function are exciting. In sharp contrast to the wealth of proven therapies for heart failure with reduced ejection fraction (HFrEF) trials of conventional heart failure medications have been inconclusive in heart failure with preserved ejection fraction (HFpEF). This is important as epidemiological studies have shown that HFpEF otherwise known as diastolic heart failure compromises half of all heart failure cases yet there is, to date, no therapy proven to reduce mortality in HFpEF (467).

1.6.3.6 Summary

In summary, the cardioprotection of SGLT2 inhibitors has been demonstrated in both human studies and in rodent models of diabetic cardiomyopathy, heart failure and myocardial ischaemia. They have been demonstrated to be effective by improving cardiac morphologic changes including LVH. They may also improve both systolic and diastolic LV function. Potential mechanisms responsible for these cardioprotective benefits of SGLT2 inhibitors and the impressive results seen in their CV outcome trials are through both systemic and direct effects and are summarised in Figure 19.

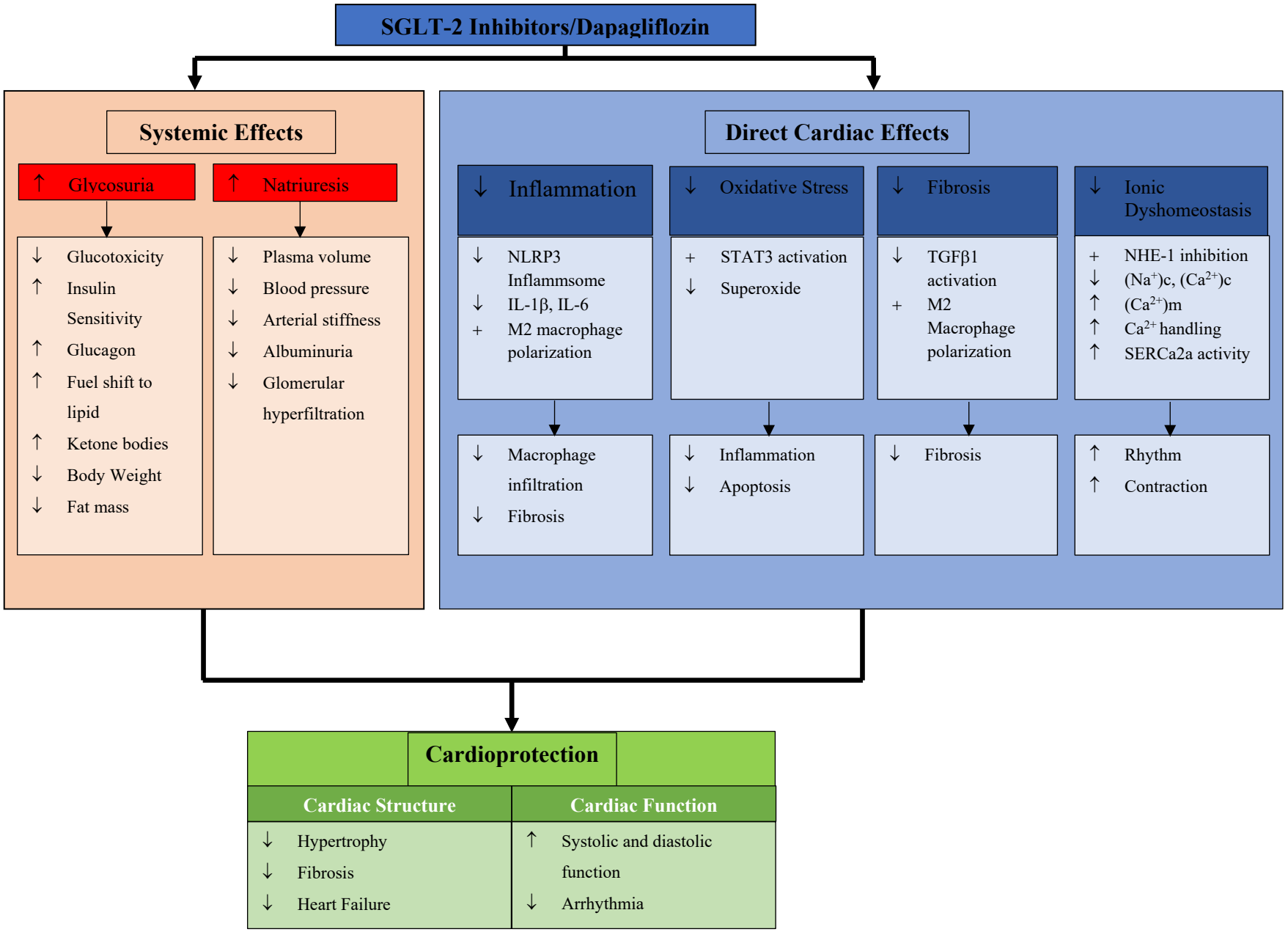


Figure 19 Summary of the proposed systemic and direct cardiac effects of SGLT2 inhibitors resulting in cardioprotection

1.7 Aims

In summary, diabetes is still a major cause of CV morbidity and mortality despite recent advances in treatments. Patients with diabetes are particularly prone to LVH, and LVH is an important, often forgotten, CV risk factor. Despite aggressive treatments for LVH with blockade of the RAS and lowering of BP, LVH still remains a problem in diabetic patients.

SGLT2 inhibitors have emerged as powerful pharmacological drugs in the prevention of heart failure. Indeed, these agents may emerge as treatment options in HFpEF and HFrEF.

The mechanisms by which these agents contribute to reduce CV events remain unclear. Specifically, whether these therapies have the ability to facilitate direct cardiac remodelling is of particular interest given the primary prevention of heart failure versus atherothrombotic events seen with these agents. The recent publication of the largest outcome study (DECLARE-TIMI 58) confirming that Dapagliflozin also significantly reduces CV death and HHF has led to the issue of how dapaglilflozin contributes to CV events to be a hot topic.

We propose that dapagliflozin may regress LVM. Dapagliflozin has a number of unique effects on the CV system which will impact on LVH. Dapagliflozin reduces BP (LV afterload) and this by itself should also further reduce LVH. Further reducing BP even in normotensive patients has been shown to definitely regress LVH. Dapagliflozin also has diuretic effects which should reduce preload on the heart (we will measure preload in this trial by MRI assessed end diastolic volume (EDV)). The fact that dapagliflozin reduces both preload and afterload on the heart makes it uniquely promising as a way to reducing future CV events in patients with diabetes and, here, in reducing LV hypertrophy. In addition, it has been shown to reduce weight and reduce insulin resistance. Therefore, dapagliflozin should regress LVH in patients with diabetes because it is unique in reducing the four main causes of LVH: glycaemia/insulin resistance, weight, preload and BP. No other anti-diabetic medication alters even three of these. Even metformin only alters two since it does not change BP. All other diabetic medications only reduce one (glycaemia) of these mediators of LVH. This may be why other new anti-glycaemic agents have failed to reduce

CV events. Furthermore, Dapagliflozin may also have a number of direct benefits on the heart by attenuating cardiac inflammation, oxidative stress, fibrosis and ionic dyshomoeostasis.

Our aim/objective of this study was therefore to establish if dapagliflozin facilitates beneficial cardiac remodelling such as regression of left ventricular hypertrophy and to study exploratory secondary outcomes such as the drug's effect on body weight and composition, BP and insulin resistance.

The outcomes of the study were

Primary Outcome:
To determine if dapagliflozin reduces LVM in patients with T2D and LVH when compared to placebo.

Secondary outcomes:

- To determine if dapagliflozin reduces LVM indexed to BSA, height, $height^{1.7}$ and $height^{2.7}$.
- To determine if dapagliflozin changes LVEDV, LVESV, LVEF and Left atrial volumes
- To determine if as expected dapagliflozin reduces blood pressure.
- To determine the effect of dapagliflozin on LV diastolic function and global longitudinal strain (GLS).
- To determine if as expected dapagliflozin reduces body weight
- To determine if dapagliflozin reduces subcutaneous and visceral fat mass and the adipokine leptin
- To determine if as expected dapagliflozin reduces $HbA1_C$ and improves insulin resistance
- To determine the effects of dapagliflozin on plasma biomarkers aimed at studying volume/wall stress, inflammation, oxidative stress and cardiac fibrosis
- To assess the tolerability of dapagliflozin in this patient group

Chapter 2 Methods

2.1 Approvals and Trial Registration

2.1.1 Ethical Approval

The clinical trial was approved by the Tayside Research Ethics Committee A, reference number 16/ES/0131.

2.1.2 Medicines and Health Regulatory Approval (MHRA)

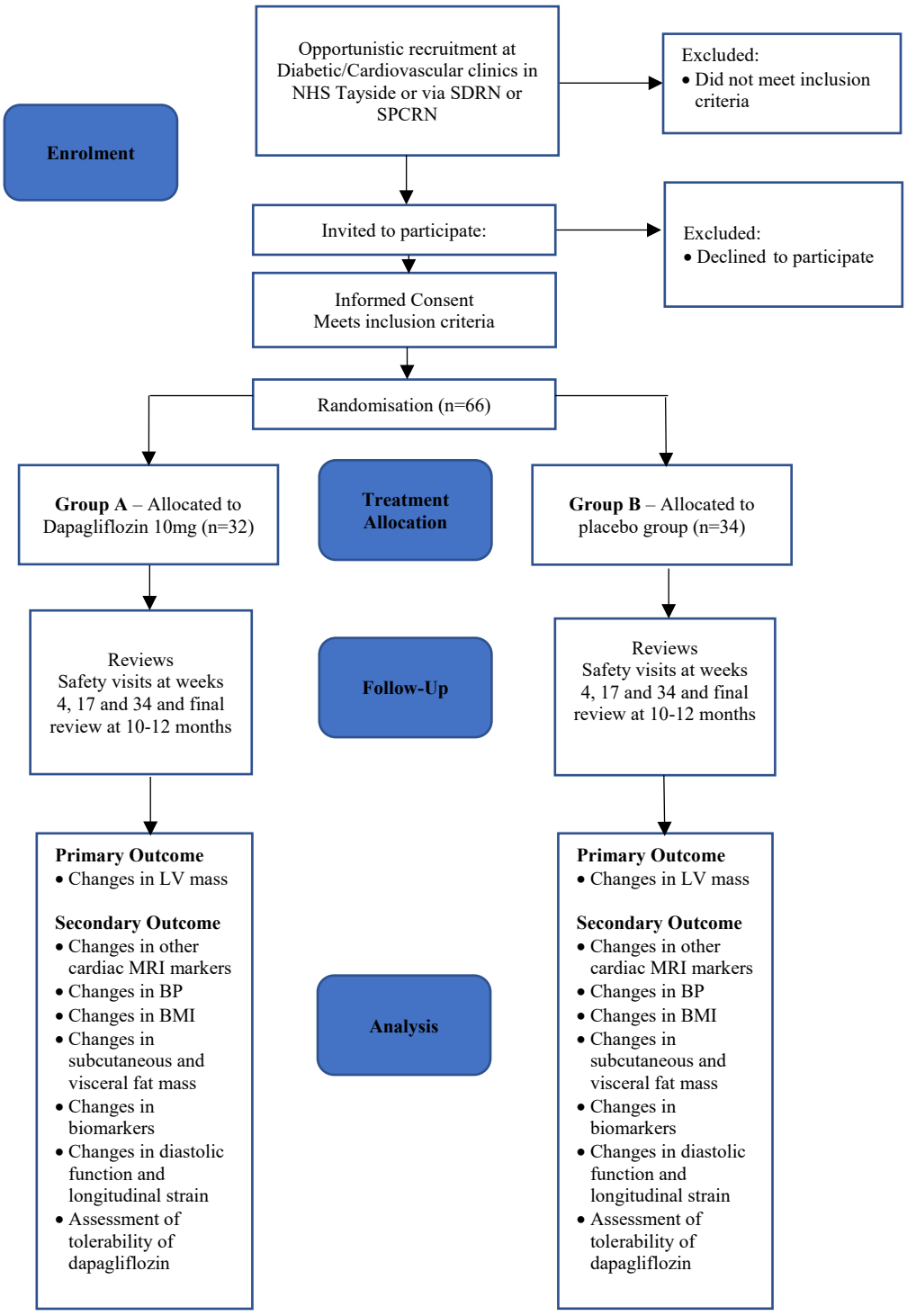
The clinical trial and use of dapagliflozin and placebo was approved by the MHRA, EudraCT number 2016-000715-33.

2.1.3 Trial Registration

This study is registered on ClinicalTrials.gov with identifier NCT02956811.

2.2 Study design

The DAPA-LVH trial was a prospective, double-blind, randomised, placebo-controlled 'proof of concept' single-centre study conducted in NHS Tayside, Scotland designed to evaluate the efficacy of 12 months of the SGLT2 inhibitor dapagliflozin compared to placebo on LVH in 66 normotensive participants with diabetes identified to have LVH. A recruitment window of 1.5 years from December 2016 was set. Participants provided written consent to participate in the study and were enrolled in this trial for a period of 10-12 months Figure 20.



SDRN Scottish Diabetes Research Network; SPCRN Scottish Primary Care Research Network; MRI Magnetic Resonance Imaging; BP Blood Pressure; Body Mass Index

Figure 20 Study design flowchart

2.2.1 Study Population

Patients were identified from a number of different sources;

- Through the Scottish Diabetes Research Network (SDRN). A search of the SDRN register will be carried out by the Research Register Manager in Tayside and Fife.
- Using the Scottish Primary Research Network (SPCRN) to identify patients via their GP in Tayside and Fife.
- Patients who have previously participated in CV research in the department and had previously consented to be contacted regards new research studies

At the screening visit an initial medical history and clinical examination was performed following informed consent. Participants had an ECG performed and bloods taken for safety analysis. Vital signs including BP were recorded to confirm eligibility prior to enrolment. BP was taken using an Omron M10-IT BP monitor and eligible patients had to have an office BP of <145/90mmHg averaged over three readings. Patients who required optimisation of their BP did so but had to be stable on their current antihypertensive medications for three months prior to enrolment. Patients with borderline office BP underwent ABPM. This was performed using a Spacelab 90217 ambulatory BP monitor. Inclusion was possible with a twenty-four hour mean BP <140/85 mmHg. Participants were also screened for echocardiographic evidence of LVH by the standard ASE criteria (30). Evaluation of LV diastolic function was also performed as per the ASE recommendations (468). This was performed using a Philips Epiq 7 machine by myself a British Society of Echocardiography accredited operator. Eligible participants identified to have LVH on echocardiography were recruited. The full inclusion criteria are listed below;

- Diagnosed with type 2 diabetes mellitus based on the current American Diabetes Association guidelines.
- Aged 18 -80 years
- Body Mass Index ≥23kg/m^2
- HbA1c 48-85mmol/mol [last known result within in the previous 6 months]

- BP <145/90mmHg. Office BP at screening visit was used however if this was above the inclusion criteria then a 24 hour recording at the screening visit was used to assess for white coat hypertension and if BP controlled the patient was deemed eligible.
- Echocardiographic LV hypertrophy (defined as either an LV mass index of >115g/m^2 for men and >95g/m^2 for women indexed to BSA or > 48g/$m^{2.7}$ or 44g/$m^{2.7}$ when indexed to height$^{2.7}$)
- Women of childbearing potential (WoCBP) who agreed to take precautions to avoid pregnancy throughout the trial and for 4 weeks after intake of the last dose.

WoCBP were defined as premenopausal women who had not been surgically sterilised or had a hysterectomy, bilateral salpingectomy or bilateral oophorectomy. Women over 45 years old, who had not had a menstrual period for at least 12 months, without an alternative medical cause, were considered post-menopausal.

2.2.2 Exclusion Criteria

Exclusion criteria included;

- Any condition that in the opinion of the investigator/myself rendered the participant unable to complete the trial including non CV disease [e.g. active malignancy].
- Participants with type 1 diabetes mellitus
- Participants who had previously had an episode of diabetic ketoacidosis.
- Serum Potassium or Sodium results outwith the normal range
- Diagnosis of clinical heart failure
- History of human immunodeficiency virus
- LV systolic dysfunction [LVEF <45%] [last known result within in the previous 6 months]
- eGFR <45ml/min/1.73m^2 [last known result within in the previous month] assessed using an abbreviated Modification of Diet in Renal Disease (MDRD) equation and indexed to 1.73m^2.

- Known liver function tests >3 times upper limit of normal [based on last measures and documented laboratory measurement in the previous 6 months]
- Body weight >150Kg [unable to fit into a MRI scanner]
- Contraindications to MRI [e.g. claustrophobia, metal implants, penetrative eye injury or exposure to metal fragments in eye requiring medical attention]
- Past or current treatment with any SGLT2 inhibitor
- Allergy to any SGLT2 inhibitor or lactose or galactose intolerance
- Current treatment with loop diuretic
- Currently receiving long term [>30 consecutive days] treatment with an oral steroid
- Pregnant or breast-feeding participants
- Involvement in the planning and/or conduct of the trial [applies to Astra Zeneca or representative staff and/or staff at the trial site].
- Participation in another interventional study [other than observational trials and registries] within 30 days before visit 1.
- Individuals considered at risk for poor protocol or medication compliance

2.2.3 Study Visits

After recruitment, patients attended for five visits over a 10-12 month period. At the randomisation visit participants had their vital signs, BMI, waist circumference and waist to hip ratio recorded. Participants also underwent 24 hour ambulatory BP monitoring using a Spacelab 90217 ambulatory monitor. Examinations with greater than 50% successful readings were deemed an acceptable exam. Bloods for safety analysis and research purposes were also be taken. All the recruited patients at the randomisation visit underwent a CMRI at the Clinical Research Centre, Ninewells Hospital, Dundee. During the visit, participants were also randomly assigned to either dapagliflozin 10mg or matching placebo as discussed in detail below. The first dose was administered during this visit and participants were educated on the symptoms of both

hypoglycaemia and diabetic ketoacidosis and given written instructions of how to manage it if either event occurred.

To reduce the likelihood of hypoglycaemia in participants taking insulin, participants who were already on insulin at time of recruitment had their total daily dose of insulin reduced by 10% on the day they were randomised. Further dose titration was done by the study team or GP based on the participant's symptoms, home and laboratory-based blood sugar concentrations. Down-titration of therapy was done in a stepwise manner starting with insulin. Other anti-diabetic agents were only down titrated once insulin had been discontinued.

In order to make the two groups comparable, a target HbA1c of ≤53mmol/mol was set for all participants. New onset diabetic patients were not included in this study as SGLT2 inhibitors are currently only licensed as second line therapy. We were therefore comparing dapagliflozin (mostly as a second drug after metformin) based group against a conventionally treated group but without a SLGT2 inhibitor.

This ensured that any difference in LV mass between groups was because dapagliflozin and all its ancillary cardiac properties and not because the two groups differed in glycaemic control.

With regards to BP, the main criteria was that the baseline office BP was <145/90mmHg. However, changes to anti-hypertensive drugs during the trial for safety reasons could occur under two circumstances. Firstly, if the systolic BP rose above 140mmHg on 2 consecutive visits during the trial then the participant was started on extra anti-hypertensive drugs to re-achieve a systolic BP of <140 mmHg. Secondly, if the participant suffered from dizziness and/or their systolic BP had fallen either by ≥25mmHg or to an absolute level of ≤110mmHg, then anti-hypertensive medications could be reduced or stopped. These criteria served two functions: firstly, to copy normal clinical practice and secondly to maintain participant safety.

There were no restrictions on clinically prescribed concomitant medications other than those listed in the exclusion criteria.

Participants returned for three review visits throughout the year to have safety bloods taken and to have vital signs, BMI, waist to hip ratio and waist circumference recorded. They were also assessed for adverse events and to alter diabetic/antihypertensive therapy if applicable.

After a minimum of 10 months, participants returned for a final visit for repeated assessment of vital signs, BMI, waist circumference and waist to hip ratio, ambulatory BP. Safety and research bloods were taken and repeat echocardiography was performed to repeat the assessment of LV

diastolic function and finally an MRI performed. These values were compared with their baseline tests to determine if any significant change has occurred within each of the two arms of the study populations. Table 7 provides an overview of all visits scheduled in the trial.

Visit	1 Screening	2 Baseline^	3 follow-up^	4 Follow-up^	5 Follow-up^	6 Last visit^	Annotations
Timeline - weeks	**0 to -4**	**0 Within 4 weeks of screening visit**	**4α (+/- 1 week)**	**17 (+/- 4 weeks)**	**34 (+/- 4 weeks)**	**(44-52) (+/- 4 weeks)**	^ Participants were fasted for these visits. α At least 3 weeks after commencing study medication # U&E, FBC, LFT, cholesterol, Lipid profile * HbA1c, FIRI, glucose, NT-proBNP, Leptin, hsCRP, Myeloperoxidase, N terminal Procollagen III peptide. A sample to be held for future genetics was also taken at the randomisation visit. Ω See section 2.2.1 ǂ The MRI scan could be performed ±3 weeks from the baseline visit and may therefore of required a separate visit although this was never required. π If the participant has not yet had their MRI scan they will be asked not to start their study medication until they have had their scan. Their Visit 3 will be delayed until the patient has had at least 3 weeks of study medication.
Informed Consent	X						
Medical History	X						
Demographics	X						
Concomitant Medications	X	X	X	X	X	X	
Physical Examination	X						
Height & weight	X						
BP & P	X	X	X	X	X	X	
Temperature	X						
ECG	X						
Echo	X					X	
Safety Bloods#	X	X	X	X	X	X	
Inclusion/Exclusion	X						
Pregnancy Testing if applicableΩ	X	X	X	X	X	X	
Research Blood Sample *		X				X	
Genetic blood sample		X					
24 hour BP		X				X	
Cardiac & abdominal MRIǂ		X				X	
Waist & hip measurement		X	X	X	X	X	
Adjustment of diabetes medication		X	X	X	X		
Adjustment of anti-hypertensive medication		X	X	X	X		
Record Adverse Events		X	X	X	X	X	
Randomisation		X					
Dispense Trial Drugs		Xπ	X	X	X		
Return trial drugs			X	X	X	X	

Table 7 An overview of all visits scheduled within the trial

2.2.4 Drug Manufacture and Randomisation

Participants were randomised to receive either dapagliflozin (10mg) or placebo in a double blind, randomised fashion. Trial medications were produced and packaged by Astra Zeneca but labelling of the packages were done by Tayside Pharmaceuticals. Randomisation was carried out via TRuST, a GCP compliant web-based system, run by the Tayside Clinical Trials Unit (TCTU), to preserve allocation concealment. The un-blinded code was held by the pharmacy department. Once randomised, the participant continued to take the trial medication once daily for a minimum of 10 months if tolerated. Compliance was checked and documented, by the dispensing pharmacy, using tablet counts at each visit. The trial was un-blinded when the database was locked, and all data had been analysed in a group only fashion.

2.2.5 Withdrawal Procedures

Participants were able to withdraw their consent to take part in the study at any point. Immediate permanent withdrawal of the trial medication occurred in the following circumstances:

1. Development of ketoacidosis
2. Serum sodium below 130 or above 149 mmol/l .
3. If serum sodium was between 130-133 or 147-149 mmol/l the test was repeated and if it remained outwith the normal range the patient was withdrawn.
4. Serum potassium below 3.2 or above 5.7 mmol/l.
5. If Serum potassium was between 3.2-3.5 or 5.4-5.7 mmol/l the test was repeated and if it remained outwith the normal range the patient was withdrawn.
6. Sustained reduction in eGFR to < 45 ml/min
7. Pregnancy
8. Participant was commenced on any prohibited medications including loop diuretics

The trial medication was also stopped temporarily for a few days, if the trial participant developed any of the following:

1. Vomiting or diarrhoea
2. The participant is effectively fasted because of nausea or any other reason.
3. The participant is undergoing surgery.
4. The patient develops pyelonephritis or urosepsis

This was because fluid resuscitation may be required in the above scenarios and the trial medication can promote hypovolaemia and therefore was only restarted once the participant was judged to be clinically euvolaemic again (396).

2.2.6 Echocardiogram

As discussed above each patient at their screening visit underwent an Echo using a Phillips Epiq 7 machine to assess for left ventricular hypertrophy as per the ASE guidelines (30). In patients enrolled in the trial diastolic left ventricular function was assessed as per the ASE guidelines. Using a apical four chamber view with colour flow imaging for optimal alignment of the pulse wave (PW) doppler with blood flow the peak E and A wave velocity were measured using a sample volume between the mitral leaflet tips. This allowed calculation of the E:A ratio and the time interval from the peak E wave along the slope of LV filling extrapolated to the zero velocity baseline allowed measurement of the mitral valve deceleration time. Using the same apical four chamber view a PW sample at the lateral and septal basal regions allowed average e^- and subsequent E:e^- ratio to be computed.

The Phillips Epiq 7 machine used in the trial was installed with Automated Cardiac Motion Quantification (aCMQ) software. This allowed the measurement of GLS which was made using the apical 4 chamber, apical 2 chamber and apical 3 chamber view. The three image loops required were selected from the stored dataset on the Philips Epiq 7 machine and the aCMQ software was applied. I visually assessed the accuracy of the software in tracking the ventricular motion and made any small manual adjustment necessary to rectify any machine misinterpretation. Only images deemed suitable to allow accurate myocardial tracking were included in the final analysis.

Patients underwent a second Echo at their final visit for repeat diastolic function and GLS assessment.

2.2.7 Cardiac and Abdominal MRI Protocol

Baseline and repeat CMR examinations at baseline visit and after a minimum of 10 months visit were performed on a 3T PrismaFIT MRI scanner [Siemens, Erlangen, Germany] using body array and spine matrix radiofrequency coils. Short axis images from the atrio-ventricular ring to the LV apex were acquired using a 2D retrospectively ECG-gated breath hold segmented SSFP cine sequence with retrospective gating. The

imaging parameters were TR/TE = 3.34/1.46 ms, and 52° flip angle, and the image stack was acquired over a typical (patient size dependent) field of view of 340-380 mm using 6mm slices. The in-plane pixel matrix was 216x256 and parallel imaging (i-PAT factor 2) was applied, along with bandwidth 977 Hz/pixel. Images were typically acquired at a rate of two slices per breath hold (scan times approximately 10-15 seconds) although this was reduced to a single slice per breath hold if a shorter scan time was necessary. The images were exported and the analysis was performed by myself using CVI 42 (Circle Cardiovascular Imaging software, Calgary, Canada). This was done for the quantitative measurement of LVM, EF, end-diastolic volume (EDV), end-systolic volume (ESV), cardiac output (CO) and stroke volume (SV) which were derived by region of interest contours placed around endocardial and epicardial LV borders at end systole and end diastole.

Papillary muscle mass was included in the LVM measurement. Care was taken to ensure that the slice ranges were matched for the baseline and final datasets even if it meant the removal of basal or apical slices to allow accurate slice matching. This will have meant an underestimation of the absolute LVM in some scans but ensured accurate comparison between the baseline and final scans. LVM and LV volumes were also indexed to body surface area, which were calculated using the Mosteller formula.

Short axis images using the same methods and pulse sequences as for the LV images were also obtained from the atrioventricular ring to the base of the atria. Left atrial volumes were derived by region of interest contours placed around the left atrial endocardial borders at end systole again using CVI 42 (Circle Cardiovascular Imaging software, Calgary, Canada). The left atrial appendage was included as part of the left atrial volume while the pulmonary veins were excluded.

Each scan was analysed at least twice and the reproducibility of LVM using CMRI was derived by myself and a test-retest intra-observer co-efficient of variation of 2.0% was used as in the departments past MRI studies. If any exceeded this co-efficient of variation for the measurement of LVM they were repeated and the final measurement was taken as the average of the two closest measurements.

After the CMRI an abdominal MRI was performed to assess subcutaneous and visceral abdominal fat mass. Scout images were acquired in order to localise the full structure of the abdomen in the sagittal, coronal and transverse planes, and a 3D dual-Echo Dixon Volume Interpolated Breath hold Examination (Dixon VIBE) sequence was

subsequently applied. The sequence was applied twice in the axial orientation covering the entire abdomen via two overlapping 3D blocks. The pulse sequence imaging parameters were TR 3.67ms, TE 1.23 and 2.46ms, flip-angle (FA) 9º and bandwidth 1040 Hz/pixel. A total of 166-192 slices (matrix size 169x320) were acquired over a field of view of 390x480 mm, and each slice was 3mm thick. Each acquisition was performed in a single breath hold (typically 10 seconds) with the patient holding their breath at end expiration.

Following the acquisition, the 'fat-only' images were visually inspected and any overlapping slices between the two 3D datasets were removed. The remaining images were then combined to form a single dataset using ImageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA) and the resulting file was exported for analysis using Analyze (Version 12.0, Mayo Clinic, Rochester, MN). The analysis was performed by a blinded MRI physicist Steve Gandy. This was done in three steps, namely (i) application of baseline regions of interest (ROI) to define sub-cutaneous adipose tissue structures (SCAT) and visceral adipose tissue structures (VAT) using a manually chosen signal-intensity threshold; (ii) selection of anterior and posterior segmentation boundaries - ranging from the top of the diaphragm to the tip of the femoral heads; and (iii) manual removal of 'non-abdomen' structures (such as the arms) together with other non-visceral structures such as bone marrow, renal cortex and similar soft-tissues. Care was taken to ensure that the slice ranges were as consistent as possible for the baseline and final datasets. Our chosen rule was to ensure that on a per-patient basis the slice ranges at each of the time-points were consistent to within 3 slices (9mm).

2.2.8 Laboratory Methods

2.2.8.1 Biochemistry and Haematology Tests

Blood samples for haemoglobin, urea and electrolytes, liver function tests, glucose, HBA1c and a full lipid profile were all analysed locally at the laboratories in Ninewells Hospital, Dundee. The samples were collected using standard vacutainer tubes and the samples stored at room temperature before immediate transfer to our laboratory.

2.2.8.2 FIRI – Fasting Insulin Resistance Index

Blood was collected and centrifuged at 3000rpm for 10 minutes then the plasma was decanted into an aliquot that was stored at -80ºC. Fasting insulin for FIRI was measured using the ALPCO Insulin an ELISA which is designed for the quantitative determination of insulin in serum and plasma.

2.2.8.3 N Terminal Brain Natriuretic Peptide (NT-proBNP)

Blood was collected and centrifuged at 3000rpm for 10 minutes then the plasma was decanted into an aliquot that was stored at -80ºC. NT-proBNP was measured using the Meso Scale Discovery Electrochemiluminescence immunoassay for human NT-pro BNP. The Human NT-proBNP Assay detects NT-proBNP in a sandwich immunoassay format.

2.2.8.4 Myeloperoxidase (MPO)

Blood was collected and centrifuged at 3000rpm for 10 minutes then the serum was decanted into an aliquot that was stored at -80ºC. MPO was measured using the R&D Systems Quantikine Human MPO Immunoassay a 4.5 hour solid-phase an enzymeELISA designed to measure human MPO in various sample types including serum.

2.2.8.5 High sensitivity CRP (hsCRP)

Blood was collected and centrifuged at 3000rpm for 10 minutes then the serum was decanted into an aliquot that was stored at -80ºC. HsCRP was measured using the Kalon High Sensitivity CRP assay (hs-CRP ELISA).

2.2.8.6 Leptin

Blood was collected and centrifuged at 3000rpm for 10 minutes then the serum was decanted into an aliquot that was stored at -80ºC. Leptin was measured using the R&D Systems Quantikine Human Leptin Immunoassay a 3.5 hour solid phase an ELISA designed to measure soluble human Leptin in serum and plasma.

2.2.8.7 N terminal Procollagen III peptide

Blood was collected and centrifuged at 3000rpm for 10 minutes then the serum was decanted into an aliquot that was stored at -80ºC. N terminal Procollagen III peptide was measured using the Cloud Clone Procollagen III N-Terminal Propeptide (PIIINP) competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of PIIINP in human serum and plasma.

2.2.9 Data Collection and Management

Data were collected by myself and documented onto a paper case record form (CRF) for subsequent transcription to the study database. Both the physical and electronic storage of the data protected the confidentiality of participants.

The data were also documented into the medical notes which acted as source data for past medical history, subsequent medical conditions, hospital admissions, diagnostic reports and blood results.

The CRF was developed by myself together with the trial management team and statistician to ensure that the data management system supported the research aims of the study. Data were stored on servers housed at the University of Dundee. Backup-up and disaster recovery was also in place.

All research related blood sample analysis from the NHS tests was stored in an unidentifiable format in a password protected disaster recovery formatted database.

Participants were informed of data storage as part of their informed consent.

Microsoft Excel was used for the data management and data management was conducted in accordance with a local standard operating procedure. The spreadsheet was designed by myself and checked by a statistician before data entry began. Data entry was carried out by myself and checked by another individual.

2.2.10 Statistical Analysis

2.2.10.1 Sample Size and power calculations

For the primary outcome of LV mass regression using cardiac MRI, the study was powered for an absolute change in LV mass based on previous studies that have been conducted in the department. One published study of LVH regression using allopurinol

in participants with ischaemic heart disease(313), found that allopurinol significantly reduced LV mass by -5·2± 5·8 grams compared to placebo -1·3±4·5 grams [p<0·007]. In per-cent terms, this degree of LVH regression was the same as seen between the two arms of the Echo sub-study of the LIFE study where CV events were also different between groups. For an 80% power at a 5% significance level [α=0·05], to detect a similar change in LV mass, we required 29 subjects per group. Previous studies in the department showed a 10% dropout rate. Therefore, accounting for this, we required a minimum total of 64 participants [32 per group]. The 10% dropout rate is standard for such studies and included those who withdrew consent.

2.2.10.2 Missing Data

The primary analysis was based on the intention-to-treat principle i.e. all participants who had baseline measurements and took at least one dose of the investigational medicinal product were analysed as part of the group to which they were randomised. The extent of missing data were examined and the reason for drop-out ascertained. Baseline observation carried forward was used to impute missing values if necessary and where assumptions for missing at random data were met. Complete case analysis where missing patients were excluded was carried out as a secondary analysis to provide a true estimate of the efficacy of intervention.

2.2.10.3 Efficacy Analyses

Data for continuous outcome measures were assessed for normality prior to analysis.

The comparison between the two groups (Dapagliflozin vs Placebo) was compared using an independent t-test for normally distributed continuous variables and a Mann Whitney test for non-normally distributed continuous variables. Categorical variables between the two treatment groups were compared using an X^2 test. Continuous variables with normal distribution were presented as mean (SD). Non-normally distributed data were presented as medians alongside their interquartile ranges (IQR).

2.2.10.4 Primary Efficacy Analysis

The primary outcome measure of LVM change was analysed as described above and presented as the between group difference in change in LVM between baseline visit and final visit. A further comparison between the two arms of the trial for LV mass was assessed by linear regression of the outcome (change in LVM) on treatment group, controlling for the baseline LV mass value and other important covariables (baseline 24 hour systolic BP, baseline 24 hour diastolic BP and ACEi and ARB prescription).

Similar analyses were carried out for the LVM indexed to BSA and height$^{1.7}$ and height$^{2.7}$.

2.2.10.5 Secondary Analyses

For most secondary endpoints between group differences in change were calculated and compared using an independent t – test, if the data were normally distributed, or a Mann Whitney test if non-normally distributed.

For weight, a repeated measures linear mixed model was used to analyse whether there was a significant difference overall between treatment groups. Independent t –tests were used to see at which time points during the study a significant difference appeared between the groups.

For 24 hour SBP, daytime SBP and nocturnal SBP the change in SBP further sensitivity analysis was performed similar to the primary endpoint. The systolic blood pressure change was the dependent variable and the baseline BP was the covariate and an analysis of covariance (one-way ANCOVA) was carried out.

A correlation between the primary end point and all the other study endpoints was also carried out.

2.2.10.6 Technical Details

All statistical analysis was carried out using IBM SPSS Statistics V22.0 (IBM, United States). A two-sided p value <0.05 was considered statistically significant. P-values ≥ 0.001 were reported to 3 decimal places; p-values less than 0.001 were reported as "<0.001".

Chapter 3 Results

3.1 Study Recruitment

From January 2017 to April 2018 a total of 1541 invitation letters were sent to potential patients. Overall 473 (31%) replied that they were interested. After reviewing their electronic medical records, 153 were excluded from invitation if eligibility criteria were not met or if the patient declined after reading the detailed information pack provided. A total of 320 participants were screened from February 2017 to May 2018. Out of the 320 patients screened 254 were excluded for the following reasons;

- 203 participants did not meet the echocardiographic criteria for LVH as per the ASE.
- 10 participants were hypertensive.
- 17 participants had an $HbA1_c$ out with the inclusion criteria.
- 5 participants declined after attending the screening visit.
- 5 participants had previously been on an SGLT2 inhibitor.
- 5 participants were found to suffer from claustrophobia.
- 9 participants had other reasons;
    - 2 participants were found to have severe LVSD.
    - 2 participants were unable to obtain holiday insurance.
    - 2 participants were on diet therapy only.
    - 1 participant worked offshore meaning they could not comply with the trial schedule.
    - 1 participant was already enrolled in another trial
    - 1 participant had recently commenced regular steroids for giant cell arteritis

66 participants were randomised. 32 participants were recruited to the Dapagliflozin arm and 34 participants to the placebo arm of the trial. Four participants withdrew from the study (3 from the Dapagliflozin group and 1 from the placebo group). The reasons for withdrawing from the trial included: breast cancer, unable to obtain holiday insurance, hyponatraemia and claustrophobia which meant the participant was reluctant to complete the final CMR. Figure 21 shows the trial CONSORT diagram.

3.2 CONSORT diagram

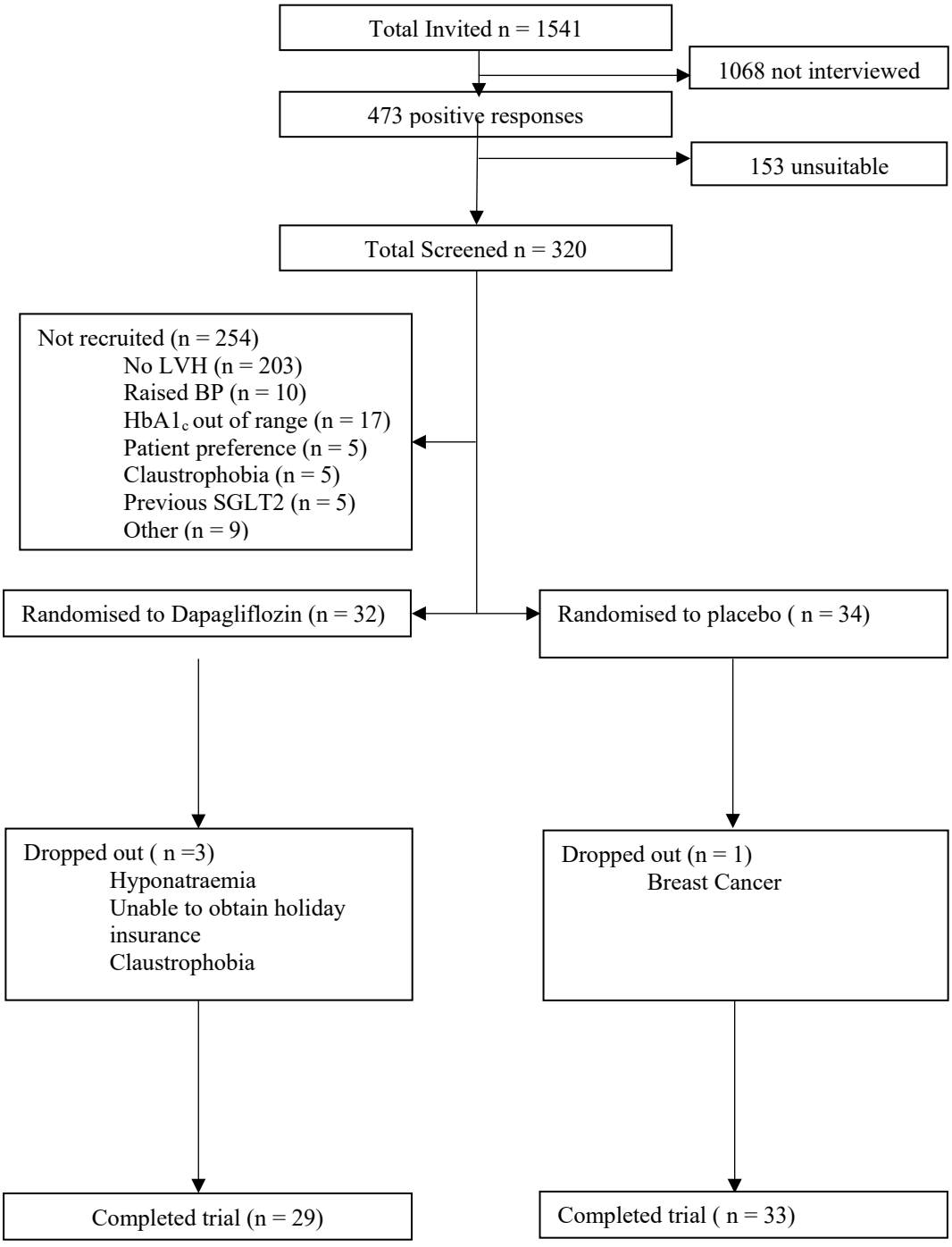


Figure 21 CONSORT diagram

3.3 Baseline Characteristics

The baseline characteristics of the participants are outlined in Tables 8-13. The mean age of the participants was 66 years. The average weight of the participants was 92kg with an average BMI of 32. The average ambulatory blood pressure of the participants was 129/73mmHg. The average LV mass measured by CMR was 124 grams and 60g/m^2 when indexed to BSA. There was no significant difference in any of the baseline characteristics between the two treatment arms. (Table 8, Table 9, Table 10, Table 11, Table 12 and Table 13)

Variable	All patients	Dapagliflozin	Placebo	P value
Total Randomised	66	32	34	
Age (years)	65.53 ± 6.87	64.25 ± 7.01	66.74 ± 6.62	0.143
Male	38 (57.6%)	20 (62.5%)	18 (52.9%)	0.432
Weight (Kg)	91.53 ± 14.26	91.58 ± 14.62	91.48 ± 14.13	0.977
BMI	32.45 ± 4.41	32.30 ± 4.66	32.59 ± 4.22	0.793
Waist Circumference (cm)	106.8 ± 10.29	105.93 ± 9.88	107.60 ± 10.74	0.514
Hip Circumference (cm)	109.24 ± 9.58	108.82 ± 10.06	109.64 ± 9.25	0.729
Waist to Hip ratio	0.98 ± 0.68	0.98 ± 0.63	0.98 ± 0.73	0.717
Office SBP Baseline (mmHg)	136.68 ± 8.32	137.25 ± 7.5	136.15 ± 9.11	0.594
Office DBP Baseline (mmHg)	78.45 ± 8.4	79.16 ± 8.63	77.79 ± 8.25	0.514

Table 8 Baseline Characteristics

Data are mean ± SD or n (%).
Abbreviations: BMI, Body Mass Index.

3.3.1.1 Past Medical history and concomitant medications

Variable	All patients	Dapagliflozin	Placebo	P value
		N=32	N=34	
IHD	8 (12.1%)	2 (6.3%)	6 (17.6%)	0.260
Hypertension	51 (77.3%)	26 (81.3%)	25 (73.5%)	0.454
Stroke	7 (10.6%)	1 (3.1%)	6 (17.6%)	0.106
Atrial Fibrillation	1 (1.5%)	1 (3.1%)	0 (0.0%)	0.485
Hypercholesterolemia	38 (57.6%)	17 (53.1%)	21 (61.8%)	0.478
Peripheral arterial disease	1 (1.5%)	1 (3.1%)	0 (0.0%)	0.485
Family History of IHD	13 (19.7%)	5 (15.6%)	8 (23.5%)	0.420
Never smoked	31 (47.0%)	14 (43.8%)	17 (50.0%)	0.611
Current Smoker	4 (6.1%)	3 (9.4%)	1 (2.9%)	0.348
Ex - Smoker	31 (47.0%)	15 (46.9%)	16 (47.1%)	0.988
*Duration of diabetes (years)	10 ± 9.0	8.5 ± 9.0	10 ± 8.0	0.343
ACE inhibitor	35 (53.0%)	17 (53.1%)	18 (52.9%)	0.988
ARB	11 (16.7%)	5 (15.6%)	6 (17.6%)	0.826
CCB	22 (33.3%)	9 (28.1%)	13 (38.2%)	0.384
Thiazide Diuretic	13 (19.7%)	9 (28.1%)	4 (11.8%)	0.095
Beta-blocker	9 (13.6%)	4 (12.5%)	5 (14.7%)	0.794
Alpha-blocker	7 (10.6%)	4 (12.5%)	3 (8.8%)	0.705
Aspirin	10 (15.2%)	4 (12.5%)	6 (17.6%)	0.734
Clopidogrel	7 (10.6%)	2 (6.3%)	5 (14.7%)	0.428
Statin	55 (83.3%)	25 (78.1%)	30 (88.2%)	0.271
Metformin	66 (100.0%)	32 (100.0%)	34 (100.0%)	Constant
Sulphonlylurea	15 (22.7%)	7 (21.9%)	8 (23.5%)	0.873
DDP-IV inhibitor	7 (10.6%)	4 (12.5%)	3 (8.8%)	0.705
GLP-1 agonist	7 (10.6%)	4 (12.5%)	3 (8.8%)	0.705
Thiazolidinedione	3 (4.5%)	0 (0.0%)	3 (8.8%)	0.239
Insulin	14 (21.2%)	7 (21.9%)	7 (20.6%)	0.898

Table 9 Past medical history and concomitant medications

Data are n (%) unless otherwise indicated. *Median ± IQR.
Abbreviations: ACE, Angiotensin Converting Enzyme; ARB, Angiotensin Receptor Blocker; CCB, Calcium Channel Blocker; DDP-IV Dipeptidyl Peptidase-4; GLP-1, Glucagon Like Peptide; IHD, Ischaemic Heart Disease.

3.3.1.2 Baseline Cardiac and Abdominal MRI Measurements

Variable	All patients	Dapagliflozin	Placebo	P value
		N=32	**N=34**	
Absolute MRI LV mass (g)	123.96 ± 22.46	126.47 ± 20.54	121.61 ± 24.20	0.383
MRI LV mass index (g/m^2)	59.95 ± 8.26	60.92 ± 7.76	59.04 ± 8.73	0.360
EF (%)	71.94 ± 5.86	71.31 ± 5.42	72.54 ± 6.27	0.398
EDV (mls)	124.04 ± 24.07	127.63 ± 22.54	120.66 ± 25.29	0.243
ESV (mls)	35.34 ± 10.63	37.17 ± 9.92	33.63 ± 11.13	0.178
SV (mls)	88.69± 17.65	90.45 ± 16.36	87.03 ± 18.88	0.435
CO (mls/min)	6186.54 ± 1143.32	6310.23 ± 1078.73	6070.12 ± 1078.74	0.398
Left atrial volume (mls)	88.42 ± 23.82	92.69 ± 27.19	84.41 ± 19.73	0.165
Left atrial area (cm^2)	23.91 ± 5.25	24.73 ± 5.86	23.13 ± 4.55	0.218
⋓Visceral adipose tissue volume (cm^3) (n=65)	6372.55 ± 2038.19	6301.79 ± 1988.24 (n=31)	6437.06 ± 2110.43	0.792
⋓ Subcutaneous adipose tissue volume (cm^3) (n=62)	9135.8 ± 3425.26	9058.34 ± 3857.04 (n=31)	9213.27 ± 2994.46 (n=31)	0.860
⋓Visceral adipose tissue volume/Subcutaneous adipose tissue Ratio (n=62)	0.77 ± 0.33	0.79 ± 0.31 (n=31)	0.74 ± 0.35 (n=31)	0.583

Table 10 Baseline Cardiac and Abdominal MRI data

Data are mean ± SD
⋓ Scans removed due to artefact and therefore unable to interpret accurately – see text for details
Abbreviations: CO, Cardiac Output; EDV, End Diastolic Volume; ESV, End Systolic Volume; EF, Ejection Fraction; LV, Left Ventricular; SV, Stroke Volume.

3.3.1.3 Baseline Ambulatory and Office Blood Pressure Measurements

Variable	All patients	Dapagliflozin	Placebo	P value
		N=32	**N=34**	
⋔24 hour SBP baseline (n=65)	129.02 ± 10.09	130.41 ± 9.62	127.67 ± 10.65	0.281
⋔24 hour DBP baseline (n=65)	73.42 ± 7.04	74.41 ± 7.88 (n=33)	72.46 ± 6.09 (n=33)	0.267
⋔ϴHeart Rate baseline (n=65)	75.31 ± 13.91	74.44 ± 13.9	76.15 ± 14.08 (n=33)	0.623
⋔Daytime SBP baseline (n=65)	131.43 ± 10.74	132.59 ± 10.37	130.30 ± 11.19 (n=33)	0.394
⋔Daytime DBP baseline (n=65)	75.37 ± 7.37	76.44 ± 8.57	74.33 ± 5.94 (n=33)	0.253
⋔⋔Nocturnal SBP baseline (n=64)	120.5 ± 12.06	123.84 ± 11.1	119.81 ± 12.8 (n=32)	0.183
⋔⋔Nocturnal DBP baseline (n=64)	67.5 ± 7.77	68.97 ± 7.84	(66.00 ± 7.52) (n=32)	0.127
Office SBP baseline	136.68 ± 8.32	137.25 ± 7.5	136.15 ± 9.11	0.594
Office DBP baseline	78.45 ± 8.4	79.16 ± 8.63	77.79 ± 8.25	0.514

Table 11 Baseline Ambulatory and Office Blood Pressure Measurements

Data are mean ± SD; BP=mmHg
⋔One patient unable to tolerate ABPM. ⋔⋔ Further patient unable to tolerate nocturnal ABPM.
ϴ Heart Rate taken from ambulatory 24 hour recording
Abbreviations: SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure.

3.3.1.4 Baseline Blood Results

Variable	All patients	Dapagliflozin	Placebo	P value
		N=32	**N=34**	
Haemoglobin (g/L)	138.36 ± 12.72	138.31 ± 13.61	138.41 ± 12.03	0.514
Haematocrit (%)	41.73 ± 3.31	41.46 ± 3.30	41.99 ± 3.35	0.975
Creatinine (umol/L)	68.11 ± 18.38	65.09 ± 16.36	70.94 ± 19.92	0.199
GFR (ml/min/1.73^2)	101.88 ± 27.06	107.53 ± 25.40	96.56 ± 27.86	0.100
Fasting glucose (mmol/L)	8.05 ± 2.96	7.80 ± 3.50	8.05 ± 3.00	0.964
Fasting Insulin	11.08 ± 11.51	10.56 ± 12.68 (n=22)	11.38 ± 11.42 (n=26)	0.521
◇*HOMA-IR (n=48)	4.03 ± 4.03	4.03 ± 4.26 (n=22)	5.03 ± 4.41 (n=26)	0.756
HbA1c (mmol/mol)	60.94 ± 10.61	61.75 ± 11.19	60.18 ± 10.15	0.551
HDL-Cholesterol (mmol/l)	1.17 ± 0.44	1.15 ± 0.41	1.22 ± 0.47	0.807
LDL-Cholesterol (mmol/l)	2.04 ± 0.73	2.12 ± 0.73	1.96 ± 0.73	0.377
Total Cholesterol (mmol/l)	4.05 ± 0.95	4.06 ± 0.85	4.04 ± 1.04	0.936
Total Cholesterol:HDL Ratio	3.44 ± 1.11	3.48 ± 1.10	3.4 ± 1.14	0.795
Triglycerides (mmol/l)	1.56 ± 1.04	1.46 ± 1.06	1.67 ± 1.47	0.186
*NT-proBNP (pg/ml)	274.42 ± 452.32	217.98 ± 477.64	365.03 ± 533.26	0.218
*Leptin (pg/ml)	15651.50 ± 23274.55	13121.05 ± 23396.73	17920.70 ± 28229.20	0.124
*Myeloperoxidase (ng/ml)	117.66 ± 181.59	129.14 ± 218.37	114.37 ± 151.36	0.837
*NT pro collagen III (ng/ml)	16.60 ± 7.32	15.91 ± 7.90	17.25 ± 7.64	0.878
*hsCRP (ng/ml)	1696.30 ± 3279.76	1168.55 ± 4049.91	2225.01 ± 3170.99	0.349

Table 12 Baseline blood results

Data are mean ± SD. * Median ± IQR
◇ Only performed on patients not on Insulin
Abbreviations: HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NT-proBNP, N-terminal pro natriuretic peptide.

3.3.1.5 Baseline Echocardiogram Measurements

Variable	All patients	Dapagliflozin	Placebo	P value
		N=32	N=34	
Echo LV mass index (g/m^2)	119.08 ± 13.48	118.81 ± 12.49	119.32 ± 14.54	0.879
Deceleration time (ms)	219.70 ± 46.62	224.18 ± 45.13	215.47 ± 48.27	0.452
Φ*E:A Ratio	0.8 ± 0.4	0.8 ± 0.3	0.8 ± 0.4	0.811
Early lateral annular tissue doppler velocity (cm/s)	7.89 ± 1.85	8.10 ± 1.98	7.50 ± 1.70	0.190
Early septal annular tissue doppler velocity (cm/s)	6.31 ± 1.20	6.43 ± 1.36	6.19 ± 1.04	0.405
*Average E/e' Ratio	9.85 ± 4.4	9.55 ± 4.4	10.3 ± 4.1	0.245
⋔Global longitudinal strain (%) (n=49)	-17.95 ± 2.04	-17.74 ± 2.10 (n=24)	-18.14 ± 2.01(n=25)	0.497

Table 13 Baseline echocardiogram values

Data are mean (SD). * Median (IQR)
ΦOne participant had atrial fibrillation therefore no A wave measurement
⋔ Scans with image quality suitable for global longitudinal strain.

3.4 Effect of Dapagliflozin on LVM and LVMI

66 patients underwent CMRI (Dapagliflozin, n=32; placebo n=34). At baseline the LVM was similar between the two groups. After an average of 11.9 months treatment in the ITTA dapagliflozin significantly reduced LVM (change in LVM: dapagliflozin group -3.95 ± 4.85g vs. placebo group -1.13 ± 4.55 g; p=0.018), leading to an absolute mean difference of -2.82g (95% confidence interval (CI): -5.13 to -0.51). The reduction on LVM was even greater in the per-protocol population (change in LVM: dapagliflozin group -4.36 ± 4.92 g vs. placebo group -1.17 ± 4.43 g; p=0.011), leading to an absolute mean difference of -3.20g (95% CI: -5.62 to -0.77).

Additional sensitivity analysis of both the ITT and per-protocol population using a one-way ANCOVA to compare the effectiveness of treatment, adjusting for relevant confounders was conducted. The covariates included in the model were baseline systolic and diastolic 24 blood pressure, baseline weight, baseline LVM and whether the participants were on an ACEi or an ARB. This also showed that the reduction in LVM remained greater in the dapagliflozin group compared to placebo: (a) for the ITT arm - estimated marginal means: dapagliflozin group, -3.98g (95% CI: -5.67, -2.29) vs

placebo group -1.11g (95% CI: -2.75, 0.54) and (b) per-protocol population - estimated marginal means: dapagliflozin group, -4.39g (95% CI: -6.21, - 2.57) vs placebo group -1.06 g (95% CI: -2.79, -0.67) and remained statistically significant (p=0.020 for ITT and p= 0.011 for per-protocol analysis), suggesting that this finding was robust and not driven by potential relevant baseline characteristics. (Table 14 and Table 15)

Dapagliflozin did not significantly reduce LVMI to BSA in either the ITT population (change in LVMI BSA: dapagliflozin group -0.58 ± 2.29 g/m^2 vs. placebo group -0.38 ± 1.79 g/m^2; p=0.691), leading to an absolute mean difference of -0.20g/m^2 (95% CI: -1.21 to 0.80). There was no reduction in LVMI BSA either in the per-protocol population (change in LVMI BSA: dapagliflozin group -0.64 ± 2.40 g/m^2 vs. placebo group -0.39 ± 1.81 g/m^2; p=0.644), leading to an absolute mean difference of -0.25g/m^2 (95% CI: -1.32 to 0.82). (Table 14, Table 15 and Figure 22)

Dapagliflozin however did result in significant reduction in LVMI to height, $height^{1.7}$ and $height^{2.7}$ in both the ITT and per protocol populations. This remained the case following sensitivity analysis correcting for the same confounders discussed above.

Variable	Intention to Treat Analysis				Per Protocol Analysis			
	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95% CI)	P Value	Dapagliflozin (n=29)	Placebo (n=33)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-3.95± 4.85	-1.13 ± 4.55	-2.82 (-5.13 to -0.51)	**0.018**	-4.36 ± 4.92	-1.17 ± 4.43	-3.2 (-5.62 to -0.77)	**0.011**
LVMI BSA (g/m^2)	-0.58± 2.29	-0.38 ± 1.79	-0.20 (-1.21 to 0.80)	0.691	-0.64 ± 2.40	-0.39 ± 1.81	-0.25 (-1.32 to 0.82)	0.644
LVMI Height (g/m)	-2.33± 2.87	-0.71 ± 2.68	-1.62 (-2.99 to -0.26)	**0.021**	-2.57 ± 2.91	-0.73 ± 2.72	-1.84 (-3.27 to -0.41)	**0.013**
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.61± 2.00	-0.51 ± 1.87	-1.09 (-2.05 to -0.15)	**0.024**	-1.78 ± 2.03	-0.52 ± 1.89	-1.25 (-2.25 to -0.25)	**0.015**
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.95± 1.20	-0.32 ± 1.12	-0.63 (-1.21 to -0.06)	**0.031**	-1.05 ± 1.22	-0.33 ± 1.14	-0.72 (-1.32 to-0.12)	**0.020**

Table 14 Changes in LVM and LVM indexed to BSA, Height, $Height^{1.7}$ and $Height^{2.7}$ after dapagliflozin treatment

P-values in bold indicate p<0.05; ^Absolute mean Difference between groups**.** All values expressed in mean ± SD unless stated.
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass: LVMI, Left Ventricular Mass Indexed.

Variable	Intention to Treat Analysis				Per Protocol Analysis			
	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95% CI)	P Value	Dapagliflozin (n=29)	Placebo (n=33)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-3.98 ± 0.85	-1.11 ± 0.82	-2.87 (-5.27 to -0.48)	**0.020**	-4.39 ± 0.91	-1.06 ± 0.86	-3.33 (-5.87 to -0.79)	**0.011**
LVMI BSA (g/m^2)	-0.58 ± 0.37	-0.37 ± 0.35	-0.23 (-1.26 to 0.80)	0.365	-0.64 ± 0.40	-0.34 ± 0.38	-0.31 (1.41 to 0.80)	0.581
LVMI Height (g/m)	-2.36 ± 0.50	-0.68 ± 0.48	-1.69 (-3.09 to -0.28)	**0.020**	-1.80 ± 0.37	-0.47 ± 0.35	-1.33 (-2.36 to -0.30)	**0.012**
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.64 ± 0.34	-0.48 ± 0.334	-1.16 (-2.13 to -0.183)	**0.021**	-1.07 ± 0.22	-0.29 ± 0.21	-0.78 (-1.39 to -0.160	**0.012**
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.98 ± 0.21	-0.30 ± 0.20	-0.68 (-1.26 to -0.10)	**0.023**	-1.07 ± 0.22	-0.29 ± 0.21	-0.78 (-1.39 to -0.160	**0.014**

Table 15 Changes in LVM and LVM indexed to BSA, Height, $Height^{1.7}$, $Height^{2.7}$ after dapagliflozin treatment following sensitivity analysis

P-values in bold indicate p<0.05; ^ Estimated marginal mean difference between groups**.** All values expressed in estimated marginal mean ± standard error unless stated.
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass: LVMI, Left Ventricular Mass Indexed.

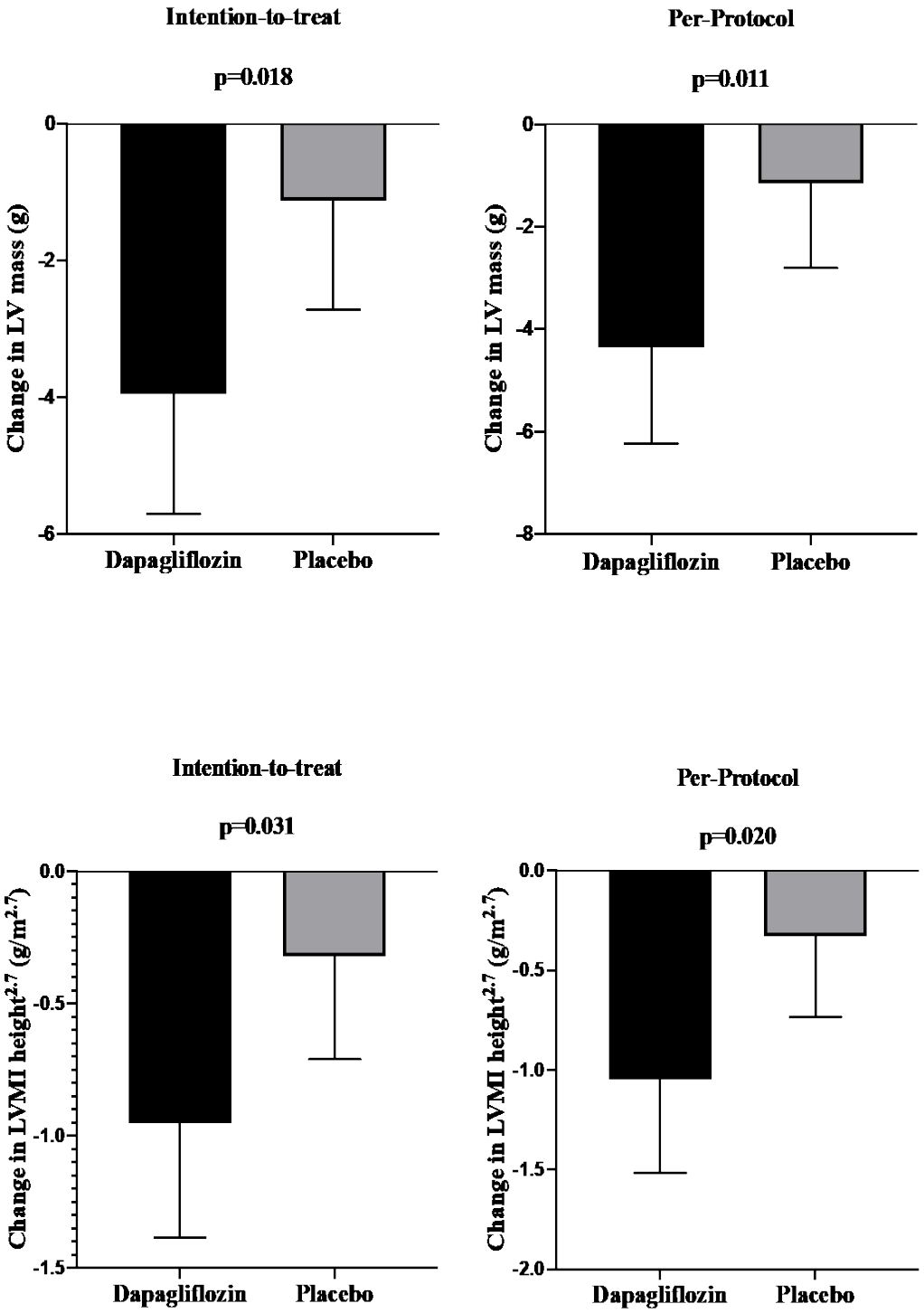


Figure 22 Column bar charts showing the mean regression of LVM and LVMi height$^{2.7}$ following dapagliflozin treatment

(Error bars represent 95% confidence interval)

3.5 Effect of Dapagliflozin on Parameters Measured on CMRI

For the other parameters measured on CMR there were no significant changes in EDV, ESV, LVEF, SV or CO. In the per protocol population there was a trend towards a reduction left atrial area with a median change in left atrial area of the dapagliflozin group -0.5 ± 3.75 cm^2 vs placebo group 0.0 ± 3.5 cm^2; p=0.088), leading to an absolute mean difference of -1.29 cm^2 (95% CI: -3.01 to 0.44). (Table 16)

LVEF did not improve in the dapagliflozin group vs the placebo group in either the ITT population (p=0.415) or the per protocol group (p=0.372).

Variable	Intention to Treat				Per Protocol			
	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95% CI)	P Value	Dapagliflozin (n=29)	Placebo (n=33)	Difference^ (95% CI)	P Value
EF (%)	1.45 ± 4.08	0.66 ± 3.76	0.79 (-1.14 to 2.72)	0.415	1.60 ± 4.26	0.68 ± 3.81	0.92(-1.13 to 2.97)	0.372
EDV (mls)	-0.15 ± 11.59	1.44 ± 10.62	-1.59 (-7.06 to 3.87)	0.562	-0.17 ± 12.20	1.48 ± 10.78	-1.65(-7.49 to 4.18)	0.573
ESV (mls)	-1.86 ± 4.83	-0.74 ± 4.81	-1.12 (-3.50 to 1.25)	0.348	-2.05 ± 5.04	-0.76 ± 4.89	-1.29(-3.82 to 1.23)	0.310
SV (mls)	1.71 ± 11.18	2.18 ± 10.45	-0.47 (-5.79 to 4.85)	0.860	1.88 ± 11.75	2.24 ± 10.60	-0.36(-6.04 to 5.32)	0.900
CO (mls/min)	-244.75 ± 1155.82	12.91 ± 865.14	-257.66 (-757.75 to 242.43)	0.307	-270.07 ± 1213.24	13.3 ± 878.55	-283.37 (-817.01 to 250.27)	0.292
Left atrial volume (mls)	-3.09 ± 10.12	-1.51 ± 8.81	-1.58(-6.24 to 3.08)	0.501	-3.41 ± 10.60	-1.56 ± 8.94	-1.16(-6.73 to 4.41)	0.458
*Left atrial area (cm^2)	-0.25 ± 3.38	0.00 ± 3.5	-1.20(-2.82 to 0.42)	0.143	-0.5 ± 3.75	0.0 ± 3.5	-1.29(-3.01 to 0.44)	0.088

Table 16 Changes in other parameters measured by CMR after dapagliflozin treatment

^Absolute mean Difference between groups**.** All values expressed in mean ± SD unless stated. *Median ±IQR
Abbreviations: CO, Cardiac Output; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; SV, Stroke Volume.

3.6 Effect of Dapagliflozin on Visceral and Subcutaneous Abdominal Tissue

In total 66 patients also underwent an abdominal MRI. In addition to the 4 withdrawals in the trial one further patient who did complete the CMR in the final visit could not complete the final abdominal MRI due to claustrophobia reducing the number of participants completing the abdominal scan to n=61.

The MR imaging was performed successfully. However, the experiment was technically complex, and the following technical issues were noted:

- The images from two participants were considered to be of sub-standard quality, based on problems associated with motion artefacts and uneven water suppression. One of these participants was omitted from the study entirely (reducing the completing participants to n=60) and in the other case it was only possible to report the VAT volume (i.e. SCAT volume was omitted).
- In n=7 of the largest participants, it was not possible to include the entire SCAT volume within the 480mm FOV. In n=2 of these cases the omitted SCAT regions were deemed large enough to render the volumes as potentially unreliable. In the remaining n=5 cases, the omitted SCAT regions were extremely small (estimated to be <5% of the total SCAT volume) and visually consistent across the two time-points. Consequently, these participants were retained within the study.
- Finally, there were n=7 cases where the radiographic overlap between the two slice blocks on the baseline data were not quite sufficient. However, it was a very simple process to adjust the 12-month data analysis in order to exactly match the included abdominal slices across both time-points.

Following careful review as described above the final cohort in the per protocol population consisted of n=60 participants where baseline and 12 month VAT volumes were available for analysis, and n=57 participants where baseline and 12 month SCAT volumes were available for analysis.

In both the ITT and the per protocol population dapagliflozin treatment significantly reduced VAT. In the ITT population (change in VAT: dapagliflozin group -565.2 ± 691.3 cm^3 vs placebo group 114.2 ± 593.7 cm^3; p<0.001), leading to an absolute mean difference of -679.4 cm^3 (95% CI: -998.0 to -360.8). (Table 17and Table 18)

Dapagliflozin treatment also resulted in both populations in a significantly reduced SCAT. In the ITT population (change in SCAT: dapagliflozin group -720.8 ± 687.8 cm^3 vs placebo group -111.1 ± 643.4 cm^3; p=0.001), leading to an absolute mean difference of -609.8cm^3 (95% CI: -948.1 to -271.3). (Table 17, Table 18 and Figure 23)
This also meant dapagliflozin significantly reduced the VAT/SCAT ratio in both the ITT (p=0.023) and the per protocol (p=0.023) populations.

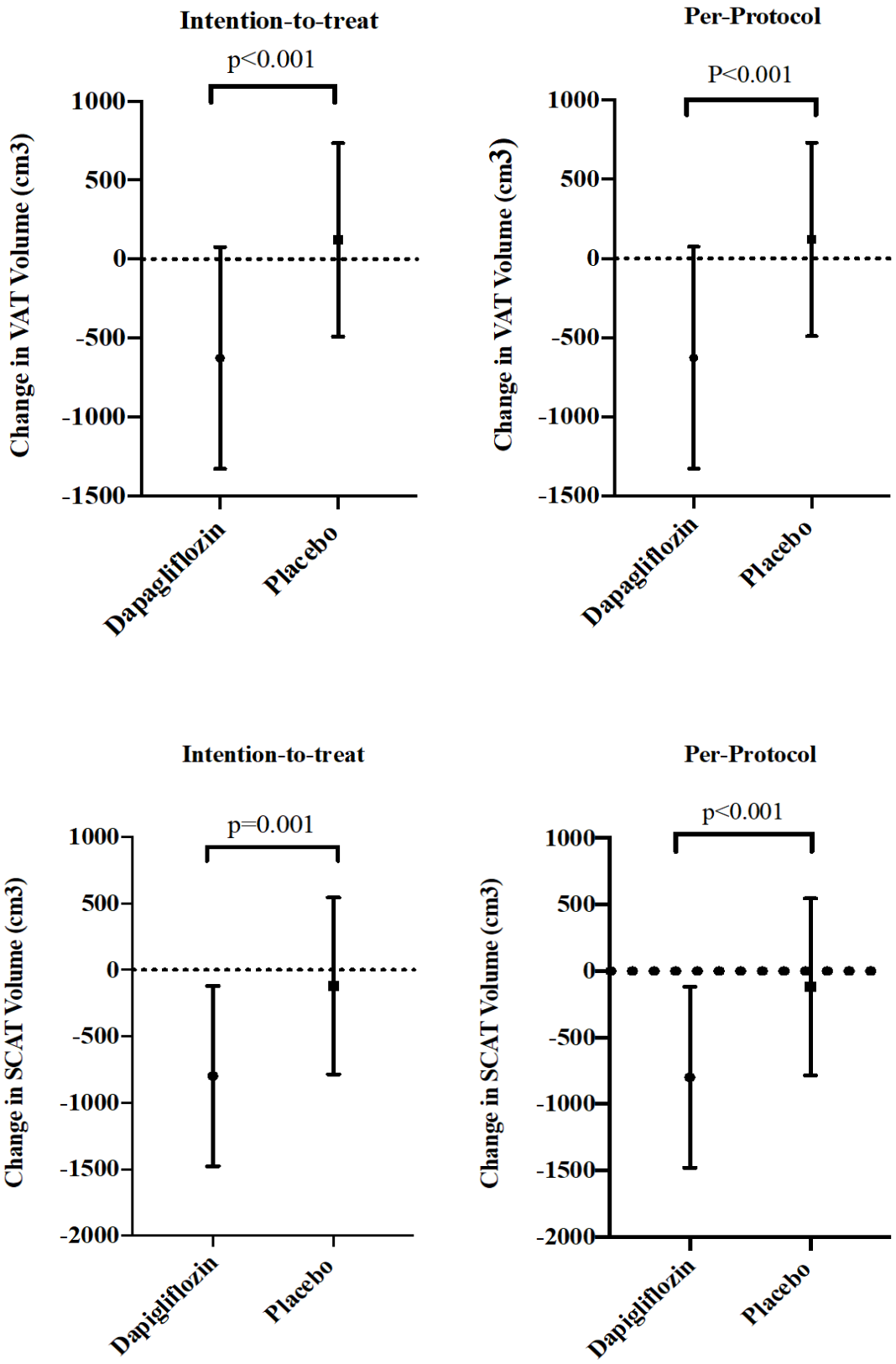


Figure 23 Column graph showing the effect of Dapagliflozin on VAT and SCAT volumes

Circles/squares represent mean change and error bars represent standard deviation
Abbreviations: SCAT, Subcutaneous Adipose Tissue; VAT, Visceral Adipose Tissue

Variable Change	Dapagliflozin (n=31)	Placebo (n=34)	Difference^ (95% CI)	P value
Visceral adipose tissue volume (cm^3) (⋔n=65)	-565.17 ± 691.27	114.22 ± 593.69	-679.40(-998.00 to -360.80)	**<0.001**
Subcutaneous adipose tissue volume (cm^3) (⋔⋔n=62)	-720.84 ± 687.83	-111.08 ± 643.42 (n=31)	-609.76(-948.13 to -271.28)	**0.001**
VAT/SCAT Ratio (⋔⋔n=62)	-0.01 ± 0.06	0.02 ± 0.06 (n=31)	-0.03(-0.06 to 0.00)	**0.023**

Table 17 Changes in VAT, SCAT and VAT/SCAT ratio after dapagliflozin treatment in the intention to treat population

⋔ One baseline scan removed due to artefact making accurate VAT measurement not possible – see text for details
⋔⋔ Further three baseline scans removed due to artefact making accurate SCAT measurement not possible – see text for details
P-values in bold indicate p<0.05; ^ Absolute mean Difference between groups**.** All values expressed in mean ± SD unless stated.
Abbreviations: SCAT, Subcutaneous Adipose Tissue; VAT, Visceral Adipose Tissue,

Variable Change	Dapagliflozin (n=28)	Placebo (n=32)	Difference^ (95% CI)	P value
Visceral adipose tissue volume (cm^3) (⋔n=60)	-625.73 ± 701.18	121.36 ± 611.81	-747.09(-1086.34 to -407.84)	**<0.001**
Subcutaneous adipose tissue volume (cm^3) (⋔⋔n=57)	-798.07 ± 679.52	-118.74 ± 665.30 (n=29)	-679.33(-1036.47 to -322.19)	**<0.001**
VAT/SCAT Ratio (⋔⋔n=57)	-0.01 ± 0.06	0.021 ± 0.057 (n=29)	-0.04(-0.07 to -0.01)	**0.023**

Table 18 Changes in VAT, SCAT and VAT/SCAT Ratio after dapagliflozin treatment in the per protocol population

⋔ One baseline scan removed due to artefact making accurate VAT measurement not possible – see text for details
⋔⋔ Further three baseline scans removed due to artefact making accurate SCAT measurement not possible – see text for details
P-values in bold indicate p<0.05; ^Absolute mean Difference between groups**.** All values expressed in mean ± SD unless stated.
Abbreviations: SCAT, Subcutaneous Adipose Tissue; VAT Visceral Adipose Tissue.

3.7 Effect of Dapagliflozin on Weight

In the ITT population dapagliflozin significantly reduced weight (change in weight: dapagliflozin group -4.3 ± 2.5 kg vs. placebo group -0.5 ± 2.3 kg; $p<0.001$), leading to an absolute mean difference of -3.8 kg (95% CI: -4.9 to -2.6). The reduction in weight was even greater in the per-protocol population (change in weight: dapagliflozin group -4.6 ± 2.4 kg vs. placebo group -0.5 ± 2.2 kg; $p<0.001$), leading to an absolute mean difference of -4.0kg (95% CI: -5.2 to -2.9). (Table 19)

Further mixed model ANOVA analysis of the per protocol population showed the weight loss effect to be significant between the first three visits of the trial (i.e. the first 4-6 months). In the latter visits the weight loss between visits was not significant. (Table 20 and Figure 24)

In both populations dapagliflozin treatment also resulted in significant reductions in BMI (ITTA, $p<0.001$; Per Protocol, $p<0.001$), waist circumference (ITTA, $p=0.001$; Per Protocol, $p=0.002$) and hip circumference (ITTA, $p<0.001$; Per Protocol, $p<0.001$). Dapagliflozin did not result in any significant change in waist to hip ratio. (Table 19)

Variable Change	Intention to Treat Analysis				Per Protocol Analysis			
	Dapagliflozin (n=32)	Placebo (n-34)	Difference^ (95% CI)	P value	Dapagliflozin (n=29)	Placebo (n=33)	Difference^ (95% CI)	P value
Weight (Kg)	-4.27 ± 2.50	-0.50 ± 2.19	-3.77(-4.92 to -2.61)	**<0.001**	-4.56 ± 2.41	-0.52 ± 2.22	-4.03(-5.21 to -2.86)	**<0.001**
BMI (Kg/m^2)	-1.53 ± 0.93	-0.17 ± 0.74	-1.35(-1.77 to -0.94)	**<0.001**	-1.63 ± 0.91	-0.18 ± 0.75	-1.45(-1.87 to -1.03)	**<0.001**
Waist Circumference (cm)	-3.23 ± 2.23	-1.39 ± 2.18	-1.83(-2.92 to -0.75)	**0.001**	-3.32 ± 2.21	-1.43 ± 2.21	-1.89(-3.02 to -0.77)	**0.001**
Hip Circumference (cm)	-3.39 ± 2.11	-1.33 ± 2.21	-2.06(-3.13 to -1.00)	**<0.001**	-3.68 ± 1.89	-1.36 ± 2.23	-2.32(-3.38 to -1.26)	**<0.001**
*Waist to Hip ratio	0.00 ± 0.04	0.01 ± 0.04	0.01(0.01 to -0.01)	0.416	-0.01 ± 0.04	-0.01 ± 0.04	0.01(-0.01 to 0.03	0.235

Table 19 Changes in Weight, BMI, Waist Circumference, Hip Circumference and Waist to Hip Ratio after dapagliflozin treatment

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. *Median ±IQR
Abbreviations: BMI, Body Mass Index

Visit (A)	Visit (B)	Weight Difference Between Visits (Kg) (A-B)	(95% CI)	P Value
2 (RV)	3	1.49 ± 0.25	(0.74 to 2.25)	**<0.001**
	4	3.6 ± 0.40	(2.37 to 4.83)	**<0.001**
	5	4.11 ± 0.50	(2.59 to 5.64)	**<0.001**
	6 (FV)	4.56 ± 0.45	(3.19 to 5.92)	**<0.001**
3	2 (RV)	1.49 ± 0.25	(-2.25 to -0.74)	**<0.001**
	4	2.11 ± 0.39	(0.93 to 2.29)	**<0.001**
	5	2.62 ± 0.46	(1.22 to 4.02)	**<0.001**
	6 (FV)	3.06 ± 0.46	(1.67 to 4.46)	**<0.001**
4	2 (RV)	-3.6 ± 0.40	(-4.83 to -2.37)	**<0.001**
	3	-2.11 ± 0.39	(-3.29 to -0.93)	**<0.001**
	5	0.51 ± 0.27	(-0.30 to 1.33)	0.655
	6 (FV)	0.96 ± 0.43	(-0.34 to 2.25)	0.327
5	2 (RV)	-4.11 ± 0.50	(-5.64 to -2.59)	**<0.001**
	3	-2.62 ± 0.46	(-4.02 to -1.22)	**<0.001**
	4	-0.51 ± 0.27	(-1.33 to 0.30)	0.655
	6 (FV)	0.44 ± 0.41	(-0.81 to 1.70)	1.000
6 (FV)	2 (RV)	-4.56 ± 0.45	(-5.91 to -3.19)	**<0.001**
	3	-3.06 ± 0.46	(-4.46 to -1.67)	**<0.001**
	4	-0.96 ± 0.43	(-2.25 to 0.34)	0.327
	5	-0.44 ± 0.412	(-1.70 to 0.81)	1.000

Table 20 Weight loss over time in the dapagliflozin arm in the per protocol population

P-values in bold indicate p<0.05;
All values expressed in estimated marginal means ± Standard error unless stated.
Abbreviations: FV, Final Visit; RV, Randomisation Visit.

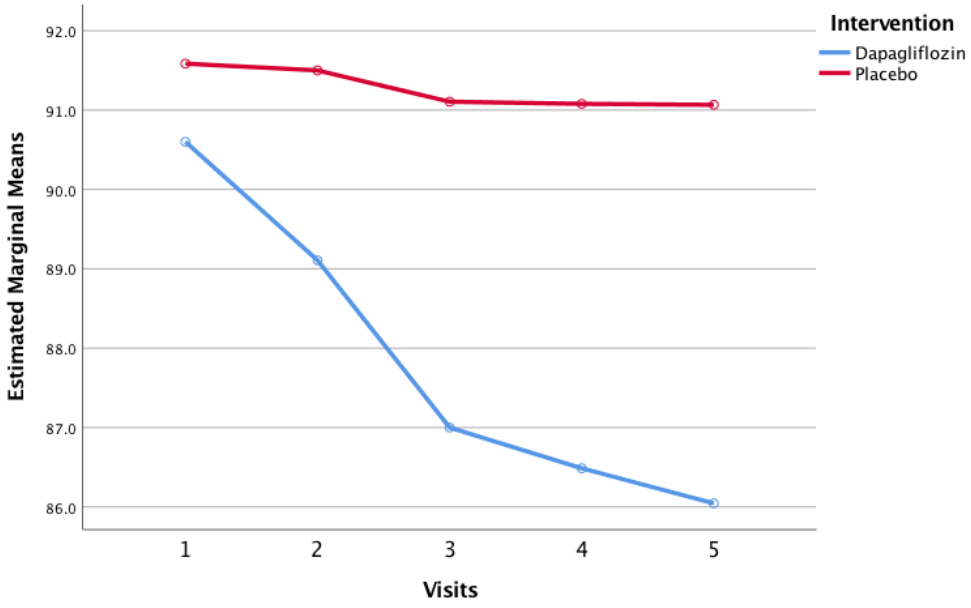


Figure 24 Changes in weight over time throughout the study with dapagliflozin treatment

3.8 Effect of Dapagliflozin on Blood Pressure

In total only one participant who completed the trial was unable to tolerate ambulatory blood pressure monitoring. A further participant was able to tolerate daytime monitoring but was unable to tolerate overnight measurements.

In both populations dapagliflozin treatment resulted in a significant reduction in 24 hour systolic blood pressure and nocturnal systolic blood pressure. In the ITT population the change in 24 hour systolic blood pressure in dapagliflozin group was -2.8 ± 5.9 mmHg vs. placebo group 0.9 ± 5.4 mmHg; p=0.012, leading to an absolute mean difference of –3.6 mmHg (95% CI: -6.4 to -0.8). The change in nocturnal systolic blood pressure was -3.5 ± 7.5 mmHg vs placebo group 0.9 ± 6.7 mmHg; p=0.017), leading to an absolute mean difference of -4.4mmHg (95% CI: -7.9 to -0.8). (Table 21)

Both these changes remained significant after additional sensitivity analysis using a one-way ANCOVA to compare the effectiveness of treatment, after adjusting for baseline blood pressure. In the ITT population the estimated marginal mean change in 24 hour systolic was -2.6 ± 1.0 mmHg vs placebo group 0.7 ± 1.0 mmHg; p=0.023, leading to an estimated mean difference of -3.2 mmHg (95% CI: -6.0 to -0.5). (Table 22 and Figure 25)

Dapagliflozin treatment did lead to a reduction in all the other measured blood pressure parameters but these were not significant. Most notably was a trend towards daytime systolic blood pressure reduction (ITT, p=0.066), (Per Protocol, p=0.060) and office systolic blood pressure reduction (ITT, p=0.080), (Per Protocol, p=0.059). (Table 21 and Table 22)

The changes in blood pressure seen were not associated with any significant change in heart rate.

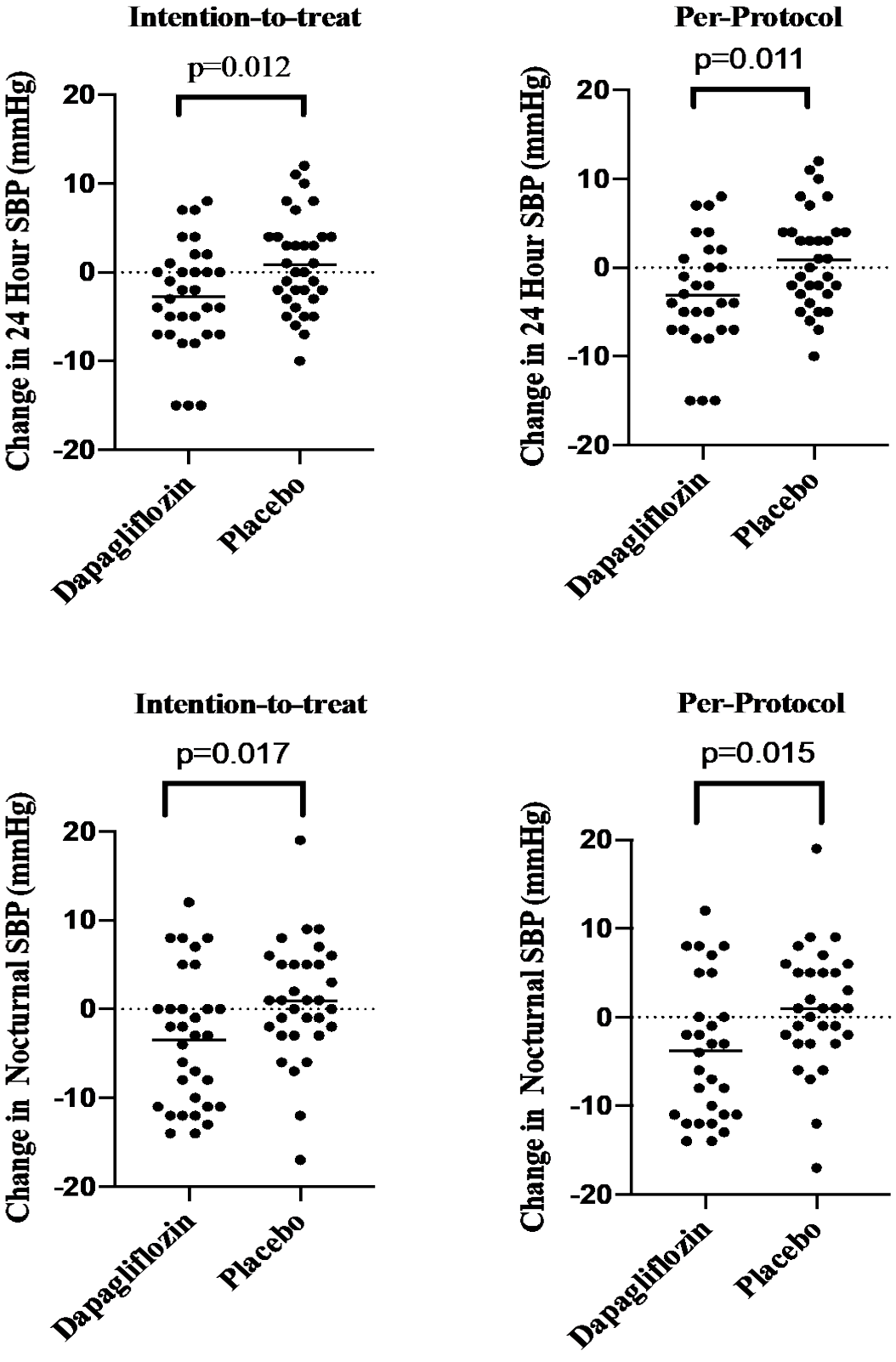


Figure 25 Scatter plot showing the change in 24 hour and nocturnal systolic blood pressure after dapagliflozin treatment

Line represents mean change

	Intention to Treat				Per Protocol			
Variable	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95%CI)	P value	Dapagliflozin (n=29)	Placebo (n=33)	Difference^ (95%CI)	P Value
⋔24 hour SBP	-2.78 ± 5.94	0.85 ± 5.40 (n=33)	-3.63(-6.44 to -0.82)	**0.012**	-3.07 ± 6.18	0.88 ± 5.48 (n=32)	-3.94(-6.93 to -0.96)	**0.011**
⋔24 hour DBP	-0.94 ± 3.98	0.06 ± 4.87 (n=33)	-0.10(-3.20 to 1.21)	0.370	-1.03 ± 4.18	0.06 ± 4.94 (n=32)	-1.1(-3.46 to 1.260	0.356
⋔*ϑHeart Rate	-2.00 ± 5.75	1.00 ± 8.50 (n=33)	-2.1(-5.64 to 1.43)	0.184	-2.0 ± 7.5	1.0 ± 8.80 (n=32)	-2.27(-6.05 to 1.51)	0.183
⋔Daytime SBP	-2.47 ± 6.56	0.55 ± 6.45 (n=33)	-3.01(-6.24 to 0.21)	0.066	-2.72 ± 6.85	0.56 ± 6.55 (n=32)	-3.29(-6.72 to 0.15)	0.060
⋔Daytime DBP	-1.03 ± 5.18	0.24 ± 5.80 (n=33)	-1.27(-4.00 to 1.46)	0.355	-1.14 ± 5.44	0.25 ± 5.90 (n=32)	-1.39(-4.30 to 1.53)	0.345
⋔⋔Nocturnal SBP	-3.47 ± 7.54	0.91 ± 6.70 (n=32)	-4.38(-7.94 to -0.81)	**0.017**	-3.83 ± 7.84	0.94 ± 6.81(n=31)	-4.76(-8.55 to -0.98)	**0.015**
⋔⋔Nocturnal DBP	-2.25 ± 5.90	0.16 ± 4.14 (n=32)	-2.41(-4.95 to 0.14)	0.063	-2.48 ± 6.16	0.16 ± 4.20 (n=31)	-2.64(-5.35 to 0.06)	0.059
Office SBP	-5.28 ± 8.63	-1.79 ± 7.26	-3.49(-7.40 to 0.43)	0.080	-5.83 ± 8.89	-1.85 ± 7.37	-3.98(-8.11 to 0.15)	0.059
Office DBP	-2.97 ± 5.62	-2.24 ± 7.48	-0.73(-4.00 to 2.54)	0.656	-3.27 ± 5.82	-2.30 ± 7.58	-0.97(-4.39 to 2.44)	0.577

Table 21 Changes in blood pressure after dapagliflozin treatment

P-values in bold indicate p<0.05; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR BP=mmHg
⋔ One participant unable to tolerate any ambulatory blood pressure monitoring, ⋔⋔ One further participant unable to tolerate overnight blood pressure monitoring.
ϑ 24 hour heart rate recorded during ambulatory blood pressure monitoring
Abbreviations: DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure

	Intention to Treat				Per Protocol			
Variable	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95% CI)	P Value	Dapagliflozin (n=29)	Placebo (n=33)	Difference^ (95% CI)	P Value
⋔ 24 hour SBP	-2.58 ± 0.98	0.66 ± 0.97 (n=33)	-3.24(-6.01 to -0.47)	**0.023**	-2.90 ± 1.45	0.72 ± 0.99 (n=32)	-3.62(-6.52 to -0.72)	**0.015**
⋔24 hour DBP	-0.79 ± 0.77	-0.09 ± 0.76 (n=33)	-0.70(-2.88 to 1.48)	0.523	-0.89 ± 0.84	-0.07 ± 0.80 (n=32)	-0.89(-2.56 to 0.78)	0.481
⋔Daytime SBP	-2.22 ± 1.09	0.31 ± 1.07 (n=33)	-2.53(-5.59 to 0.53)	0.104	-2.54 ± 1.16	0.40 ± 1.1 (n=32)	-2.94(-6.15 to 0.26)	0.071
⋔Daytime DBP	-0.84 ± 0.96	0.06 ± 0.94 (n=33)	-0.89(-3.59 to 1.81)	0.511	-0.95 ± 1.43	0.08 ± 0.98 (n=32)	-1.04(-3.91 to 1.83)	0.472
⋔⋔Nocturnal SBP	-3.12 ± 1.23	0.56 ± 1.23 (n=32)	-3.68(-7.17 to -0.19)	**0.039**	-3.51 ± 1.31	0.64 ± 1.27 (n=31)	-4.15(-7.83 to -0.47)	**0.028**
⋔⋔ Nocturnal DBP	-1.89 ± 0.85	-0.20 ± 0.85 (n=32)	-1.69(-4.12 to 0.75)	0.171	-2.14 ± 0.91	-0.16 ± 0.88 (n=31)	-1.98(-4.55 to 0.58)	0.127
Office SBP	-5.08 ± 1.31	-1.99 ± 1.27	-3.09(-6.75 to 0.57)	0.096	-5.60 ± 1.39	-2.05 ± 1.30	-3.55(-7.36 to 0.27)	0.068
Office DBP	-2.79 ± 1.12	-2.40 ± 1.09	-0.39(-3.52 to 2.74)	0.804	-3.07 ± 1.20	-2.48 ± 1.12	-0.59(-3.88 to 2.69)	0.720

Table 22 Changes in blood pressure after dapagliflozin treatment after adjustment for baseline measurements

P-values in bold indicate p<0.05; ^ Estimated marginal mean difference between groups**.** All values expressed in estimated marginal mean ± standard error unless stated.
⋔ One participant unable to tolerate any ambulatory blood pressure monitoring, ⋔⋔ One further participant unable to tolerate overnight blood pressure monitoring.
Abbreviations: DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure

3.9 Effect of Dapagliflozin on Blood Parameters

Dapagliflozin treatment resulted in a significant change in a number of safety blood parameters compared to placebo treatment in both the ITT and per protocol analysis. Dapagliflozin resulted in a significant increase in both haemoglobin and haematocrit. In the ITT analysis dapagliflozin resulted in a median increase of 7.0 ± 11.8 g/l vs placebo -2.0 ± 5.0 g/l; $p<0.001$, with an absolute mean difference of -9.5g/l (95% CI: 5.9 to 13.2). Haematocrit increased in the dapagliflozin arm by 2.6 ± 0.02 % vs placebo 0.3 ± 0.02 %; $p<0.001$, leading to an absolute mean difference of 2.9% (95% CI: 1.8 to 4.0).

Dapagliflozin treatment resulted in a significant reduction in both fasting glucose and $HbA1_c$. In the ITT analysis $HbA1_c$ in the dapagliflozin arm fell by 6.3 ± 8.3mmol/mol vs placebo -0.8 ± 10.9 mmol/mol; $p<0.025$, with an absolute mean difference of -5.5 mmol/mol (95% CI: -10.3 to 0.7). Fasting glucose significantly dropped in the dapagliflozin arm by -1.1 ± 2.1 mmol/l vs placebo 0.6 ± 2.1 mmol/l; $p=0.002$, with an absolute mean difference of -1.7mmol/l (95% CI: -2.7 to -0.7) in the ITT analysis. (Table 23)

Variable	Intention to Treat Analysis			
	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95%CI)	P Value
Haemoglobin (g/L)	7.00 ± 11.75	-2.00 ± 5.00	9.51(5.85 to 13.18)	**<0.001**
Haematocrit (%)	2.60 ± 0.02	0.30 ± 0.02	2.90(1.84 to 3.96)	**<0.001**
Creatinine (umol/L)	1.34 ± 5.89	-0.91 ± 5.83	2.26(-0.63 to 5.14)	0.123
eGFR (ml/min/1.73^2)	-1.16 ± 10.48	1.59 ± 7.19	-2.74(-7.14 to 1.65)	0.217
Fasting glucose (mmol/L)	-1.06 ± 2.08	0.62 ± 2.11	-1.68(-2.71 to -0.65)	**0.002**
HbA1c (mmol/mol)	-6.28 ± 8.25	-0.79 ± 10.89	-5.49(-10.26 to -0.71)	**0.025**
*Total Cholesterol (mmol/mol)	-0.10 ± 0.32	-0.17 ± 0.48	-0.07(-0.28 to 0.15)	0.995
*HDL-Cholesterol (mmol/l)	0.06 ± 0.19	0.00 ± 0.10	0.08(0.00 to 0.15)	**0.031**
LDL-Cholesterol (mmol/mol)	-0.14 ± 0.31	-0.08 ± 0.45	-0.06(-0.26 to 0.13)	0.522
*Total Cholesterol:HDL Ratio	0.20 ± 0.48	0.10 ± 0.42	-0.21(-0.43 to 0.01)	0.085
*Triglycerides (mmol/l)	-0.11 ± 0.31	0.01 ± 0.61	-0.17(-0.41 to 0.08)	0.064

Table 23 Changes in safety blood parameters in the intention to treat analysis after dapagliflozin treatment

P-values in bold indicate p<0.05; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR
Abbreviations: eGFR, estimated glomerular filtration rate; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein

In addition to the reduction in glucose and HbA1c the research blood analysis showed that dapagliflozin treatment also resulted in a significant reduction in insulin resistance. In the ITT analysis dapagliflozin led to a median reduction in HOMA-IR of -2.1 ± 2.4 vs placebo 0.5 ± 3.2; p=0.017, with an absolute mean difference of -2.6 (95% CI: -4.5 to -0.6).

This was associated with a trend reduction in fasting insulin in the dapagliflozin arm although this did not reach significance (p=0.098).

Dapagliflozin also led to a significant reduction in hsCRP. In the ITTA dapagliflozin resulted in a median reduction of -163.7 ± 1040.8 ng/l vs placebo 66.7± 1258.4 ng/l; p =0.049, with a mean difference of -1296.0 (95% CI: -2650.59 to -31.50).

Dapagliflozin treatment resulted in no significant change in NT-proBNP, Leptin, Myeloperoxidase or NT pro collagen. (Table 24)

Variable	Intention to Treat Analysis			
	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95%CI)	P Value
*NT-proBNP (pg/ml)	7.14 ± 138.69	40.19 ± 219.47	-103.68(-326.90 to 119.54)	0.551
*Leptin (pg/ml)	-447.55 ± 5299.58	477.60 ± 6314.88	-2931.70 7.28(-6901.46 to 1038.07)	0.256
*Myeloperoxidase (ng/ml)	0.00 ± 107.04	-36.49 ± 85.63	23.02(-31.05 to 77.08)	0.172
NT pro collagen III (ng/ml)	-0.44 ± 5.06	-0.10 ± 4.24	-0.46(-2.20 to 1.29)	0.653
*hsCRP (ng/l)	-163.73 ± 1040.76	66.73 ± 1258.37	-1296.04(-2650.59 to -31.50)	**0.049**
◇*Fasting Insulin (uU/ml) (n=48)	-2.34 ± 5.59 (n=22)	-0.58 ± 7.14 (n=26)	-3.61(-6.97 to -0.26)	0.098
◇*HOMA-IR (n=48)	-1.29 ± 2.36 (n=22)	-0.22 ± 3.23 (n=26)	-2.56(-4.47 to -0.65)	**0.017**

Table 24 Changes in research blood parameters in the intention to treat analysis after dapagliflozin treatment

P-values in bold indicate p<0.05; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR
◇ Only performed on the participants not on Insulin
Abbreviations: HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hsCRP, High Sensitive C-Reactive Protein; NT-proBNP, N-Terminal Pro Natriuretic B-Type Natriuretic Peptide

3.10 Effect of Dapagliflozin on Echocardiographic Parameters

All 66 patients underwent a baseline echocardiogram. Echocardiography was technically challenging in some of the participants. On review 15 were deemed not have images of sufficient quality to allow accurate strain analysis and therefore were excluded from this analysis.

Dapagliflozin resulted in no significant change in any of the diastolic function parameters in either the ITT or per protocol analysis. Dapagliflozin however did result in a significant improvement in global longitudinal strain. In the ITTA dapagliflozin treatment resulted in a mean increase in global longitudinal strain of -1.64 ± 2.5% vs placebo -0.2 ± 1.8; p=0.024, with a mean difference of -1.4% (95% CI: -2.7 to -0.2). (Table 25)

Variable	Intention to Treat Analysis				Per Protocol Analysis			
	Dapagliflozin (n=32)	Placebo (n=34)	Difference^ (95% CI)	P Value	Dapagliflozin (n=29)	Placebo (n=33)	Difference^ (95% CI)	P Value
Deceleration time (ms)	-8.47 ± 57.34	4.18 ± 44.90	-12.64 (-37.89 to 12.60)	0.321	-9.34 ± 60.27	4.30 ± 45.59	-13.65 (-40.60 to 13.31)	0.315
ΦΦ*E:A Ratio	0.00 ± 0.2 (n=31)	0.0 ± 0.2	0.05 (-0.5 to 0.14)	0.587	0.00 ± 0.20	0.00 ± 0.20	0.04 (-0.06 to 0.15)	0.591
Early lateral annular tissue doppler velocity (cm/s)	0.74 ± 2.37	0.49 ± 1.80	0.25 (-0.78 to 1.28)	0.635	0.81 ± 2.48	0.51 ± 1.83	0.31 (-0.79 to 1.41)	0.577
Early septal annular tissue doppler velocity (cm/s)	0.56 ± 2.22	0.26 ± 1.36	0.30 (-0.60 to 1.20)	0.503	0.10 ± 3.05	0.10 ± 2.40	0.35 (-0.61 to 1.31)	0.745
*Average E/e' Ratio	0.0 ± 2.0	-0.1 ± 3.1	-0.15 (-1.40 to 1.10)	0.621	-0.31 ± 2.43	-0.14 ± 2.77	-0.18 (-1.51 to 1.56)	0.791
ΦGlobal longitudinal strain (%)	-1.64 ± 2.51 (n=24)	-0.21 ± 1.75 (n=25)	-1.43 (-2.67 to -0.19)	**0.024**	- 1.71 ± 2.53 (n=23)	- 0.22 ± 1.78 (n=24)	-1.49 (-2.78 to -0.21)	**0.024**

Table 25 Changes in echocardiographic parameters after dapagliflozin treatment

P-values in bold indicate p<0.05; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR
Φ Cases removed as images not adequate for accurate strain analysis. ΦΦ One participant had atrial fibrillation therefore no E:A Ratio measurement

3.11 Adverse Events

There were 5 serious adverse events during the trial. One was recorded as possibly related to the study medication.

2 serious adverse events occurred in the dapagliflozin arm;

- Fall (down a flight of stairs)
- Jaw fracture

The jaw fracture was as a cause of the fall down a flight of stairs and was recorded as possibly related to the study drug as the exact cause of the fall leading to the jaw fracture was never identified. On admission to hospital however the participant was not hypoglycaemic, hypotensive and had no postural drop in blood pressure on assessment.

3 serious adverse events occurred in the placebo arm;

- Breast Cancer – required patient to be removed from the trial
- Ulnar Collateral Ligament Rupture following mechanical fall
- Haematemesis

In total there were 169 adverse events, 86 events in the dapagliflozin arm and 83 in the placebo arm. Table 26 displays the likely causality to the IMP for each arm based on the summary of product characteristics.

Causality	Dapagliflozin	Placebo
Unrelated	32	49
Possible	12	13
Probable	3	0
Definite	39	21

Table 26 Adverse Events Causality to Study Medications

Aside from the two withdrawals for the serious adverse events discussed above no participants withdrew due to side effects. Table 27 illustrates adverse events by organ system class as per the medical dictionary for regulatory activities (MedDRA) coding.

System Organ Class	Dapagliflozin	Placebo
Gastrointestinal disorders	10	10
Glucose metabolism disorders (inc diabetes mellitus)	22	7
Renal and urinary disorders	11	8
Metabolism and nutrition disorders	5	4
Injury, poisoning and procedural complications	4	3
Respiratory, thoracic and mediastinal disorders	9	16
Musculoskeletal and connective tissue disorders NEC	7	8
Infections and infestations	13	9
Nervous system disorders	1	3
Skin and subcutaneous tissue disorders	0	1
Gastrointestinal motility and defaecation conditions	1	3
Blood and Lymphatic system disorder	1	0
Psychiatric Disorders	1	0
Ear and Labyrinth disorder	1	1
Neoplasms benign, malignant and unspecified (including cysts and polyps)	0	1
General disorders and administration site conditions	0	4
Vascular disorders	0	1
Cardiac Disorders	0	3
Investigations	0	1

Table 27 Adverse Events by System Organ Class

The incidence of common side effects reported with SGLT2 inhibitors is illustrated in Table 28. There were significantly more episodes of reported hypoglycaemia and thrush in the dapagliflozin arm. There were significantly more urinary tract infections in the placebo arm although one participant in the placebo arm had three separate urinary tract infections. They were referred for

further investigation and found to have bladder emptying issues. There no reported episodes of diabetic ketoacidosis/bone fracture/thromboembolism or CVA events

Side Effect	All patients	Dapagliflozin	Placebo	P value
*Urinary Symptoms	15	8	7	0.434
^Confirmed UTI	7	1	6	**0.011**
Thrush	14	12	2	**0.046**
Dizzy/Postural Hypotension	1	0	1	0.378
$ Reported Hypoglycaemia	29	22	7	**0.059**
Thirst	4	2	2	0.631
Constipation	6	4	2	0.814
Serious Adverse Event	5	2	3	0.357

Table 28 Common SGLT2 inhibitor side effects in the two treatment arms

*Polyuria/Urinary Frequency
^Positive mid specimen stream urine
$ Reported hypoglycaemia by patient based on symptoms and or confirmed by bm monitoring

3.12 Antihypertensive and Diabetic Medication Changes

Changes to all anti-hypertensive medications is demonstrated in Table 29. There were only four changes so accurate cross tabulation was not possible. However, the small numbers suggest they were unlikely to have altered the results of the study in particular LVM.

	Dapagliflozin	Placebo
New/Up titration of antihypertensive	0	1
Stopped/Down titration of antihypertensive	2	1

Table 29 Changes in antihypertensives

In total there 48 changes to the participants diabetic medications. This does not include the protocol stipulation that all patients on insulin had their dose reduced by 10% at the randomisation visit. In total there were 14 patients on insulin with 7 in each arm, so this mandatory change was excluded from cross tabulation analysis. There was a significant increase in the number of diabetic medications increased in the placebo arm and a trend towards more medications being decreased in the dapagliflozin arm. (Table 30) Despite this there was still a significant reduction in both fasting plasma glucose and HbA1c in the dapagliflozin arm.

Diabetic Meds	All patients	Dapagliflozin	Placebo	P value
New Medication	7	3	4	0.864
Medication Increased	9	1	8	**0.028**
Medication Stopped	6	3	3	1.000
*Medication Decreased	26	15	11	0.073

Table 30 Changes in diabetic medication

3.13 Drug Compliance

This was assessed by counting the medications returned at each visit during the trial. There was no significant difference in medical compliance between those taking dapagliflozin and placebo. The significant reduction in fasting plasma glucose and HbA1c is also consistent with good compliance with dapagliflozin. (Table 31)

	Dapagliflozin	Placebo	P Value
Compliance (%)	96.5 ± 4.5	95.9 ± 5.2	0.629

Table 31 Record of drug compliance

3.14 Subgroup Analysis

To look at whether any subgroups might have benefited more from dapagliflozin the participants were stratified by high/low baseline LVMI, high/low LVEF, high/low baseline weight, high/low baseline ambulatory 24 hour and nocturnal systolic blood pressure, high/low baseline weight and high/low baseline VAT and SCAT and high/low baseline $HbA1_C$, Insulin, HOMA-IR and hsCRP. The study was not powered to evaluate subgroups, but these post-hoc sub-group analyses were performed to help explain the effects of dapagliflozin on LVM and ensure the data were fully explored.

3.15 Effect of Dapagliflozin on Cardiac MRI Parameters Above and Below Median LVMI to Body Surface Area

The median for LVMI was 60.4 g/m^2. The effect of dapagliflozin on LVM was concentrated in the group with the above-median LVMI at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -5.1 ± 4.8g vs placebo 1.1 ± 4.5g; p=0.029, leading to an absolute mean difference of -3.9g (95% CI: -7.3 to -0.4). This was in contrast to a non-significant reduction in the below median LVMI at baseline where dapagliflozin resulted in a change in LVM of -2.4 ± 4.6g vs placebo -1.1 ± 4.5g; p=0.381, leading to an absolute mean difference of -1.4g (95% CI: -4.7 to 1.8).

There remained no significant effect on cardiac volume or LVEF with dapagliflozin therapy in either subgroup. There was however a trend reduction in left atrial area with a median reduction in left atrial area in the above median LVMI subgroup of 1.25 ± 3.1 cm^2 in the dapagliflozin arm vs placebo 0.0cm^2 ± 2.0 cm^2 ; p=0.029. (Table 32)

Variable	LVMI to BSA Above Median				LVMI to BSA Below Median			
	Dapagliflozin (n=18)	Placebo (n=15)	Difference^ (95% CI)	P Value	Dapagliflozin (n=14)	Placebo (n=19)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-5.11 ± 4.87	-1.23 ± 4.8	-3.88 (-7.33 to -0.43)	**0.029**	-2.46 ± 4.57	-1.05± 4.47	-1.41 (-4.65 to 1.83)	0.381
LVMI BSA (g/m^2)	-1.02 ± 1.94	-0.39 ± 1.78	-0.64 (-1.97 to 0.69)	0.336	-0.01 ± 2.64	-0.38± 2.64	0.36 (-1.22 to 1.95)	0.644
LVMI Height (g/m)	-2.97 ± 2.79	-0.73 ± 2.70	-2.24 (-4.20 to -0.28)	**0.026**	-1.50 ± 2.86	-0.69± 2.74	-0.81 (-2.82 to 1.19)	0.415
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-2.03 ± 1.9	-0.50 ± 1.82	-1.53 (-2.86 to -0.20)	**0.025**	-1.06 ± 2.07	-0.51± 1.95	-0.55 (-1.99 to 0.89)	0.441
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-1.19 ± 1.10	-0.30 ± 1.04	-0.89 (-1.65 to -0.12)	**0.025**	-0.65± 1.31	-0.34± 1.21	-0.31 (-1.21 to 0.58)	0.481
EF (%)	1.61 ± 4.32	-0.40 ± 4.69	2.01 (-1.19 to 5.21)	0.210	1.25 ± 3.89	1.50± 2.66	-0.25 (-2.57 to 2.07)	0.828
EDV (mls)	-2.36 ± 11.49	-2.40 ± 10.19	0.04 (-7.74 to 7.83)	0.991	2.68 ± 11.51	4.47± 10.2	-1.80 (-9.53 to 5.94)	0.639
ESV (mls)	-2.67 ± 5.94	-0.70 ± 6.49	-1.97 (-6.43 to 2.50)	0.371	-0.82 ± 2.74	-0.76± 3.12	-0.06 (-2.19 to 2.08)	0.956
SV (mls)	0.31 ± 11.00	-1.70 ± 11.21	2.01 (-5.93 to 9.95)	0.608	3.50 ± 11.56	5.24± 8.94	-1.74 (-9.40 to 5.92)	0.630
CO (mls/min)	-372.97 ± 1009.91	-298.00 ± 945.91	-74.97 (-774.83 to 624.88)	0.828	-79.89 ± 1341.56	258.37± 729.48	-338.26 (-1079.16 to 402.64)	0.359
Left atrial volume (mls)	-3.81 ± 11.11	-0.43 ± 9.37	-3.37 (-10.64 to 3.90)	0.359	-2.18 ± 9.03	-2.37± 8.50	0.19 (-6.08 to 6.46)	0.951
*Left atrial area (cm^2)	-1.25 ± 3.13	0.00 ± 2.00	-1.95 (-4.02 to 0.12)	0.086	0.00 ± 3.25	0.00± 4.5	-0.06 (-2.58 to 2.43)	0.733

Table 32 Effect of Dapagliflozin on Measured Parameters on Cardiac MRI Analysed as Subgroups Above and Below Median LVMI to BSA

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR
Abbreviations: CO, Cardiac Output; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed; Stroke Volume

3.16 Effect of Dapagliflozin on Cardiac MRI Parameters Above and Below Median LVEF

The median for LVEF was 72.3%. The effect of dapagliflozin on LVM was concentrated in the group with the above-median LVEF at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -4.1 ± 4.6g vs placebo -0.1 ± 4.6g; p=0.021, leading to an absolute mean difference of -4.0g (95% CI: -7.4 to -0.7). (Table 33)

There remained no significant effect on cardiac volume or LVEF with dapagliflozin therapy in either subgroup.

Variable	LVEF above Median				LVEF below Median			
	Dapagliflozin (n=13)	Placebo (n=20)	Difference^ (95% CI)	P Value	Dapagliflozin (n=19)	Placebo (n=14)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-4.15± 4.61	-0.13± 4.66	-4.02(-7.40 to -0.66)	**0.021**	-3.82± 5.14	-2.57± 4.11	-1.24(-4.64 to 2.16)	0.461
LVMI BSA (g/m^2)	-0.84± 2.23	0.03± 1.90	-0.87(-2.32 to 0.61)	0.238	-0.40± 2.36	-0.97± 4.11	0.57(-0.90 to 2.03)	0.436
LVMI Height (g/m)	-2.58± 2.82	-0.10± 2.76	-2.48(-4.51 to -0.46)	**0.018**	-2.15± 2.97	-1.57± 1.48	-0.58(-2.55 to 1.39)	0.552
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.85± 2.01	-0.08± 1.92	-1.77(-3.19 to -0.35)	**0.016**	-1.44± 2.03	-1.12± 2.4	-0.32(-1.67 to 1.03)	0.630
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-1.16± 1.25	-0.06± 1.15	-1.09(-1.96 to -0.23)	**0.015**	-0.81± 1.19	-0.69± 1.66	-0.12(-0.92 to 0.68)	0.759
EF (%)	0.46± 4.54	0.30± 3.40	0.16(-2.90 to 3.22)	0.915	2.13±3.70	1.18±3.46	0.95(-1.63 to 3.54)	0.458
EDV (mls)	-1.85± -1.85	3.53± 10.37	-5.37(-13.84 to 3.09)	0.205	1.01± 10.38	-1.54± 0.99	2.54(-4.99 to 10.07)	0.497
ESV (mls)	-1.19± -1.19	0.33± 4.62	-1.52(-4.78 to 1.75)	0.351	-2.32± 5.24	-2.25± 10.63	-0.07(-3.71 to 3.57)	0.971
SV (mls)	-0.65± 13.56	3.20± 11.59	-3.85(-12.86 to 5.15)	0.389	3.32± 9.27	0.71± 4.83	2.61(-3.90 to 9.12)	0.420
CO (mls/min)	-399.58± 1010.51	35.28± 976.68	-434.85 (-1154.12 to 284.42)	0.227	-138.82± 1261.28	-19.04± 8.76	-119.78 (-885.11 to 645.54)	0.752
Left atrial volume (mls)	-2.34± 3.07	1.43± 2.51	-1.78(-7.89 to 4.34)	0.558	-3.84± 11.17	-3.36± 3.46	-0.48(-8.05 to 7.08)	0.897
*Left atrial area (cm^2)	-2.00± 3.25	1.00± 3.25	-3.77(-5.76 to -1.78)	0.100	0± 2.5	-0.50± 9.57	1.22(-1.21 to 3.66)	0.314

Table 33 Effect of Dapagliflozin on Measured Parameters on Cardiac MRI Analysed as Subgroups Above and Below Median LVEF

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR
Abbreviations: CO, Cardiac Output; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed; Stroke Volume

3.17 Effect of Dapagliflozin on Left Ventricular Mass Above and Below Median Ambulatory 24 Hour Systolic and Nocturnal Blood Pressure

The median 24 hour ambulatory systolic blood pressure was 129 mmHg. The effect of dapagliflozin on LVM was greatest in the group with the above-median systolic blood pressure at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -3.6 ± 5g vs placebo -0.4 ± 4.4g; p=0.057, leading to an absolute mean difference of -3.2g (95% CI: -6.5 to 0.1). (Table 34)

The median nocturnal systolic blood pressure was 121 mmHg. There was no significant change in LVM measurements in either subgroup with dapagliflozin treatment. (Table 35)

Variable	24 hour Ambulatory SBP Above Median				24 hour Ambulatory SBP below Median			
	Dapagliflozin (n=16)	Placebo (n=16)	Difference^ (95% CI)	P Value	Dapagliflozin (n=16)	Placebo (n=17)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-4.31± 4.82	-1.97± 4.67	-2.34(-5.77 to 1.08)	0.173	-3.59± 5.02	-0.39± 4.43	-3.20(-6.51 to 0.10)	0.057
LVMI BSA (g/m^2)	-0.67± 1.88	-0.63± 1.87	-0.03(-1.39 to 1.32)	0.961	-0.50± 2.69	-0.16± 1.73	-0.34(-1.90 to 1.22)	0.660
LVMI Height (g/m)	-2.52± 2.82	-1.22± 2.82	-1.30(-3.33 to 0.74)	0.203	-2.14± 3.00	-0.25± 2.55	-1.89(-3.83 to 0.05)	0.056
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.73± 1.95	-0.88± 1.99	-0.85(-2.27 to 0.57)	0.230	-1.49± 2.11	-0.18± 1.74	-1.30(-2.65 to 0.04)	0.057
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-1.02± 1.16	-0.55± 1.22	-0.47(-1.32 to 0.39)	0.277	-0.89± 1.29	-0.11± 1.01	-0.77(-1.57 to 0.03)	0.060

Table 34 Effect of Dapagliflozin on Measured Parameters on Cardiac MRI Analysed as Subgroups Above and Below Median 24 Hour Systolic Blood Pressure

In both tables P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed.

Variable	Nocturnal Ambulatory SBP Above Median				Nocturnal Ambulatory SBP below Median			
	Dapagliflozin (n=17)	Placebo (n=15)	Difference^ (95% CI)	P Value	Dapagliflozin (n=15)	Placebo (n=17)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-4.03± 5.01	-1.00± 4.75	-3.03(-6.57 to 0.51)	0.091	-3.87± 4.85	-1.15± 4.72	-2.72(-6.18 to 0.74)	0.119
LVMI BSA (g/m^2)	-0.48± 2.50	-0.14± 1.81	-0.34(-1.94 to 1.26	0.666	-0.70± 2.10	-0.51± 1.86	-0.19(-1.62 to 1.24)	0.791
LVMI Height (g/m)	-2.35± 2.99	-0.63± 2.79	-1.72(-3.81 to 0.38)	0.105	-2.30± 2.84	-0.71± 2.79	-1.59(-3.62 to 0.45)	0.121
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.61± 2.09	-0.46± 1.94	-1.15(-2.62 to 0.31)	0.119	-1.61± 1.96	-0.51± 1.94	-1.09(-2.51 to 0.32)	0.124
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.94± 1.27	-0.29± 1.16	-0.65(-1.53 to 0.24)	0.144	-0.96± 1.17	-0.32± 1.17	-0.64(-1.49 to 0.20)	0.132

Table 35 Effect of Dapagliflozin on Measured Parameters on Cardiac MRI Analysed as Subgroups Above and Below Median Nocturnal Systolic Blood Pressure

In both tables P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated. * Median ± IQR
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed.

3.18 Effect of Dapagliflozin on Left Ventricular Mass Above and Below Median Weight, VAT and SCAT Volumes

The median for weight was 90.9kg. The effect of dapagliflozin on LVM was concentrated in the group with the above-median weight at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -4.3 ± 5.9g vs placebo -0.2 ± 4.4g; p=0.029, leading to an absolute mean difference of -4.1g (95% CI: -7.8 to -0.5). (Table 34)

The median VAT volume was 6012.6cm^3. The effect of dapagliflozin on LVM was concentrated in the group with the above-median VAT at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -5.0 ± 5.1g vs placebo -0.5 ± 4.3g; p=0.002, leading to an absolute mean difference of -5.5g (95% CI: -8.8 to -2.2). Indeed, in this subgroup there was also a trend reduction in LVMI BSA where dapagliflozin resulted in a change of -1.0 ±2.0 g/m^2 vs placebo 0.2 ± 1.6 g/m^2; p =0.051, leading to an absolute mean difference of -1.3 g/m^2 (95%CI: -2.5 to 0.0). (Table 35)

The median SCAT volume was 8324.9cm^3. Conversely the effect of dapagliflozin on LVM was concentrated in the group with the below-median SCAT at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -5.1± 3.8g vs placebo -1.0 ± 4.9g; p=0.011, leading to an absolute mean difference of -4.1g (95% CI: -7.2 to -1.0).

Variable	Weight above Median				Weight below Median			
	Dapagliflozin (n=15)	Placebo (n=18)	Difference^ (95% CI)	P Value	Dapagliflozin (n=17)	Placebo (n=16)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-4.33± 5.92	-0.19± 4.43	-4.14(-7.81 to -0.46)	**0.029**	-3.62± 3.84	-2.19± 4.58	-1.43(-4.43 to 1.57)	0.338
LVMI BSA (g/m^2)	-0.73± 2.29	0.06± 1.65	-0.79(-2.20 to 0.61)	0.257	-0.45± 2.34	-0.87± 1.86	0.43(-1.08 to 1.94)	0.566
LVMI Height (g/m)	-2.48± 3.40	-0.16± 2.56	-2.32(-4.44 to -0.21)	**0.033**	-2.19± 2.41	-1.32± 2.76	-0.87(-2.70 to 0.97)	0.343
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.69± 2.32	-0.13± 1.76	-1.55(-3.00 to -0.10)	**0.036**	-1.54± 1.75	-0.93± 1.95	-0.61(-1.92 to 0.70)	0.351
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.97± 1.35	-0.1± 1.04	-0.87(-1.72 to -0.02)	**0.044**	-0.93± 1.11	-0.57± 1.19	-0.37(-1.18 to 0.45)	0.367

Table 36 Effect of Dapagliflozin on Left Ventricular Mass Analysed as Subgroups Above and Below Median Weight

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated.

Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed.

Variable	VAT above Median				VAT below Median			
	Dapagliflozin (n=15)	Placebo (n=17)	Difference^ (95% CI)	P Value	Dapagliflozin (n=16)	Placebo (n=17)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-5.00± 5.06	0.50± 4.34	-5.50(-8.84 to -2.16)	**0.002**	-3.03± 4.62	-2.97± 4.16	-0.06(3.19 to 3.07)	0.969
LVMI BSA (g/m^2)	-1.04± 2.00	0.22± 1.57	-1.26(-2.53 to 0.00)	0.051	-0.18± 2.50	-1.06± 1.81	0.88(-0.68 to 2.44)	0.257
LVMI Height (g/m)	-2.92± 2.90	0.28± 2.45	-3.20(-5.10 to -1.30)	**0.002**	-1.81± 2.83	-1.82± 2.56	0.02(-1.90 to 1.93)	0.987
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-2.01± 1.98	0.19± 1.64	-2.20(-3.48 to -0.91)	**0.001**	-1.26± 2.01	-1.30± 1.83	0.04(-1.33 to 1.41)	0.955
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-1.18± 1.15	0.11± 0.93	-1.28(-2.02 to -0.52)	**0.001**	-0.75± 1.25	-0.80± 1.14	0.05(-0.80 to 0.90)	0.908

Table 37 Effect of Dapagliflozin on Left Ventricular Mass Analysed as Subgroups Above and Below Median VAT Volume

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated.
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass

Variable	SCAT above Median				SCAT below Median			
	Dapagliflozin (n=15)	Placebo (n=16)	Difference^ (95% CI)	P Value	Dapagliflozin (n=16)	Placebo (n=15)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-2.78± 5.62	-1.26± 4.34	-1.52(-5.07 to 2.04)	0.391	-5.13± 3.76	-1.00± 4.87	-4.13(-7.23 to -1.02)	**0.011**
LVMI BSA (g/m^2)	0.06± 2.54	-0.26± 1.65	0.31(-1.20 to 1.83)	0.675	-1.22± 1.87	-0.5± 1.96	-0.72(-2.07 to 0.64)	0.291
LVMI Height (g/m)	-1.66± 3.36	-0.78± 2.62	-0.87(-3.01 to 1.26)	0.410	-3.00± 2.18	-0.63± 2.82	-2.37(-4.17 to -0.57)	**0.012**
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.16± 2.36	-0.56± 1.86	-0.59(-2.10 to 0.91)	0.428	-2.06± 1.50	-0.46± 1.93	-1.61(-2.84 to _0.37)	**0.012**
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.69± 1.44	-0.35± 1.14	-0.34(-1.26 to 0.58)	0.458	-1.21± 0.89	-0.29± 1.13	-0.92(-1.65 to -0.20)	**0.014**

Table 38 Effect of Dapagliflozin on Left Ventricular Mass Analyses as Subgroup Above and Below Median SCAT Volume

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated.
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed

3.19 Effect of Dapagliflozin on Left Ventricular Mass Above and Below Median Glycated Haemoglobin and HOMA-IR

The median $HbA1_C$ was 58mmol/mol. The effect of dapagliflozin on LVM was greatest in the group with the below-median $HbA1_C$ at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -4.5 ± 5.1g vs placebo -0.9 ± 4.1g; p=0.043, leading to an absolute mean difference of -3.6g (95% CI: -7.1to -0.1). (Table 39)

The median HOMA-IR was 4.1 and there was essentially no difference in the effect of dapagliflozin on LVM measurements in each subgroup. (Table 40)

Variable	$HbA1_C$ above Median				$HbA1_C$ below Median			
	Dapagliflozin (n=20)	Placebo (n=16)	Difference^ (95% CI)	P Value	Dapagliflozin (n=12)	Placebo (n=16)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-3.60± 4.78	-1.38± 5.04	-2.23(-5.56 to 1.11)	0.185	-4.54± 5.13	-0.92± 4.19	-3.63(-7.12 to -0.13)	**0.043**
LVMI BSA (g/m^2)	-0.30± 2.37	-0.38± 1.78	0.08(-1.38 to 1.53)	0.916	-1.04± 2.16	-0.38± 1.85	-0.66(-2.17 to 0.09)	0.376
LVMI Height (g/m)	-2.16± 2.89	-0.77± 2.93	-1.39(-3.37 to 0.60	0.165	-2.61± 2.94	-0.65± 2.52	-1.96(-4.02 to 0.10)	0.061
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.51± 2.05	-0.52± 2.02	-0.99(-2.38 to 0.39)	0.155	-1.77± 2.00	-0.50± 1.78	-1.27(-2.69 to 0.16)	0.079
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.91± 1.26	-0.30± 1.20	-0.62(-1.45 to 0.22)	0.146	-1.02± 1.15	-0.34± 1.08	-0.68(-1.54 to 0.19)	0.114

Table 39 Effect of Dapagliflozin on Left Ventricular Mass Analyses as Subgroup Above and Below Median $HbA1_C$

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated.
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed

Variable	HOMA-IR above Median				HOMA-IR below Median			
	Dapagliflozin (n=15)	Placebo (n=18)	Difference^ (95% CI)	P Value	Dapagliflozin (n=17)	Placebo (n=16)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-3.79± 4.40	-1.42± 4.18	-2.37(-5.92 to 1.18)	0.181	-4.12± 5.55	-1.89± 4.93	-2.22(-6.37 to 1.93)	0.281
LVMI BSA (g/m^2)	-0.85± 1.85	-0.32± 1.5	-0.53(-1.92 to 0.85)	0.435	-0.41± 2.91	-0.84± 1.88	0.43(-1.49 to 2.36)	0.647
LVMI Height (g/m)	-2.23± 2.53	-0.86± 2.39	-1.37(-3.40 to 0.67)	0.178	-2.37± 3.28	-1.17± 2.89	-1.19(-3.64 to 1.25)	0.325
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.54± 1.72	-0.61± 1.63	-0.93(-2.32 to 0.45)	0.178	-1.61± 2.28	-0.84± 2.00	-0.76(-2.46 to 0.93)	0.363
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.92± 1.01	-0.37± 0.95	-0.54(-1.35 to 0.27)	0.181	-0.92± 1.37	-0.52± 1.19	-0.40(-1.42 to 0.62)	0.427

Table 40 Effect of Dapagliflozin on Left Ventricular Mass Analyses as Subgroup Above and Below Median HOMA-IR

P-values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed

3.20 Effect of Dapagliflozin on Left Ventricular Mass Above and Below Median High Sensitive C- Reactive Protein

The median hsCRP was 1696.30 ng/ml. The effect of dapagliflozin on LVM was greatest in the group with the below-median hs-CRP at baseline. In this sub-group in the ITT analysis dapagliflozin resulted in a change in LVM of -4.2 ± 4.9g vs placebo -0.8 ± 4.0g; p=0.038, leading to an absolute mean difference of -3.4g (95% CI: -6.6 to -0.2). (Table 41)

Variable	hsCRP above Median				hsCRP below Median			
	Dapagliflozin (n=15)	Placebo (n=18)	Difference^ (95% CI)	P Value	Dapagliflozin (n=17)	Placebo (n=16)	Difference^ (95% CI)	P Value
Absolute LVM (g)	-3.73± 4.93	-1.47± 5.09	-2.26(-5.84 to 1.32)	0.207	-4.15± 4.93	-0.75± 3.98	-3.40(-6.59 to -0.21)	**0.038**
LVMI BSA (g/m^2)	-0.41± 1.99	-0.52± 2.02	0.11(-1.32 to 1.54)	0.878	-0.73± 2.57	-0.23± 1.53	-0.51(-2.02 to 1.01)	0.499
LVMI Height (g/m)	-2.22± 2.90	-0.86± 2.95	-1.36(-3.45 to 0.73)	0.194	-2.42± 2.93	-0.53± 2.43	-1.89(-3.81 to 0.03)	0.053
LVMI $Height^{1.7}$ ($g/m^{1.7}$)	-1.55± 2.01	-0.59± 2.03	-0.95(-2.39 to 0.49)	0.187	-1.66± 2.05	-0.42± 1.72	-1.25(-2.60 to 0.10)	0.069
LVMI $Height^{2.7}$ ($g/m^{2.7}$)	-0.93± 1.20	-0.35± 1.20	-0.58(-1.43 to 0.28)	0.179	-0.97± 1.24	-0.29± 1.06	-0.69(-1.51 to 0.14)	0.099

Table 41 Effect of Dapagliflozin on Left Ventricular Mass Analyses as Subgroup Above and Below Median High Sensitive C-Reactive Protein

In both tables p values in bold indicate $p<0.05$; ^Absolute mean Difference between groups
All other values expressed in mean ± SD unless stated
Abbreviations: BSA, Body Surface Area; LVM, Left Ventricular Mass; LVMI, Left Ventricular Mass Indexed

3.21 Correlations

There was an observed moderate correlation between change in LVM and change in ambulatory 24 SBP and nocturnal SBP with r=0.415, n= 61, p=0.001, and r=0.321, n=60, p=0.012 respectively.

There was an observed strong correlation between change in LVM and change in VAT, r= 0.592, n = 60, p<0.001, and a moderate correlation between change in LVM and change in SCAT r=0.360, n=57, p 0.006.

There was a moderate correlation between change in LVM and left atrial area and left atrial volume change, but this did not reach statistical significance. There was no significant correlation between LVM change and HOMA-IR, hsCRP or Insulin change. (Table 42)

Variable		Change LVM (g)
EDV Change (mls)	Pearson Correlation	0.205
	Sig. (2-tailed)	0.111
	N	62
ESV Change (mls)	Pearson Correlation	0.225
	Sig. (2-tailed)	0.079
	N	62
SV Change (mls)	Pearson Correlation	0.110
	Sig. (2-tailed)	0.394
	N	62
CO Change (mls/min)	Pearson Correlation	0.266
	Sig. (2-tailed)	0.037
	N	62
EF Change (%)	Pearson Correlation	-0.051
	Sig. (2-tailed)	0.695
	N	62
LVESAV Change (mls)	Pearson Correlation	0.298
	Sig. (2-tailed)	0.019
	N	62
LAA Change (cm^2)	Pearson Correlation	0.281
	Sig. (2-tailed)	0.027
	N	62
GLS Change (%)	Pearson Correlation	0.075
	Sig. (2-tailed)	0.615
	N	47
SCAT Volume Change (cm^3)	Pearson Correlation	0.380
	Sig. (2-tailed)	0.003
	N	61
VAT Volume Change (cm^3)	Pearson Correlation	0.594
	Sig. (2-tailed)	0.000
	N	61
Leptin Change (ng/ml)	Pearson Correlation	-0.09
	Sig. (2-tailed)	0.489
	N	62
Myeloperoxidase Change (ng/ml)	Pearson Correlation	0.163
	Sig. (2-tailed)	0.205
	N	62
NT pro collagen III (ng/ml)	Pearson Correlation	0
	Sig. (2-tailed)	0.997

	N	62
hsCRP Change (ng/ml)	Pearson Correlation	0.163
	Sig. (2-tailed)	0.205
	N	62
NT-proBNP Change (pg/ml)	Pearson Correlation	0.005
	Sig. (2-tailed)	0.970
	N	62
Haemoglobin Change (g/l)	Pearson Correlation	-0.291
	Sig. (2-tailed)	0.022
	N	62
Haematocrit Change (%)	Pearson Correlation	-0.229
	Sig. (2-tailed)	0.074
	N	62
Creatinine Change (umol/L)	Pearson Correlation	-0.013
	Sig. (2-tailed)	0.922
	N	62
Calculated GFR Change (ml/min/1.73^2)	Pearson Correlation	0.049
	Sig. (2-tailed)	0.708
	N	62
HbA1c Change (mmol/mol)	Pearson Correlation	0.374
	Sig. (2-tailed)	0.003
	N	62
Total Cholesterol Change (mmol/mol)	Pearson Correlation	-0.171
	Sig. (2-tailed)	0.184
	N	62
HDL Cholesterol Change (mmol/mol)	Pearson Correlation	-0.034
	Sig. (2-tailed)	0.792
	N	62
LDL Cholesterol Change (mmol/mol)	Pearson Correlation	-0.189
	Sig. (2-tailed)	0.141
	N	62
TC:HDL Ratio Change (mmol/mol)	Pearson Correlation	-0.149
	Sig. (2-tailed)	0.249
	N	62
Triglyceride Change (mmol/mol)	Pearson Correlation	0.032
	Sig. (2-tailed)	0.807
	N	62
24 Hour Systolic ABPM Change (mmHg)	Pearson Correlation	0.415
	Sig. (2-tailed)	0.001
	N	61
24 Hour Diastolic ABPM Change (mmHg)	Pearson Correlation	0.129

	Sig. (2-tailed)	0.322
	N	61
Daytime Systolic BP Change (mmHg)	Pearson Correlation	0.306
	Sig. (2-tailed)	0.016
	N	61
Daytime Diastolic BP Change (mmHg)	Pearson Correlation	-0.025
	Sig. (2-tailed)	0.848
	N	61
Nocturnal Systolic BP Change (mmHg)	Pearson Correlation	0.321
	Sig. (2-tailed)	0.012
	N	60
Nocturnal Diastolic BP Change (mmHg)	Pearson Correlation	0.233
	Sig. (2-tailed)	0.073
	N	60
BMI Change (g/m^2)	Pearson Correlation	0.506
	Sig. (2-tailed)	0.000
	N	62
Waist Circumference Change (cm)	Pearson Correlation	0.312
	Sig. (2-tailed)	0.013
	N	62
Hip Circumference Change (cm)	Pearson Correlation	0.471
	Sig. (2-tailed)	0.000
	N	62
Waist to Hip Ratio Change	Pearson Correlation	-0.259
	Sig. (2-tailed)	0.042
	N	62
HOMA-IR Change	Pearson Correlation	0.222
	Sig. (2-tailed)	0.130
	N	48
Insulin Change (uU/ml)	Pearson Correlation	0.173
	Sig. (2-tailed)	0.239
	N	48

Table 42 Correlation of Left Ventricular Mass Change with Other Measured Parameters

P-values in bold indicate $p<0.05$
Abbreviations: ABPM, Ambulatory Blood Pressure Monitoring; BP, Blood Pressure; CO, Cardiac Output; EDV, End Diastolic Volume; EF, Ejection Fraction; ESV, End Systolic Volume; SV, Stroke Volume; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hsCRP, High Sensitive C- Reactive Protein; GLS, Global Longitudinal Change; NT-proBNP, N-terminal pro natriuretic peptide. SCAT, Subcutaneous Adipose Tissue; VAT Visceral Adipose Tissue

Chapter 4 Discussion

The main finding of this study is that a 10mg once daily dose of dapagliflozin treatment significantly reduces LVM in people with T2D and LVH with blood pressure within the normal range. We also found that dapagliflozin significantly reduced measures of obesity, 24 hour ambulatory and nocturnal systolic blood pressure, glycated haemoglobin, insulin resistance and hsCRP which may explain why we observed a reduction in LVM with dapagliflozin treatment as will be discussed below.

4.1 Possible Mechanisms Why Dapagliflozin Reduces Left Ventricular Mass

4.1.1 Effect on Blood Pressure

As discussed, blood pressure is strongly implicated in the development of LVH. Dapagliflozin and SGLT2 inhibitors could mediate LVH regression through its effect on BP. Trials have consistently shown that SGLT2-inhibitors lead to a reduction in systolic BP in the range of 3-5mmHg in patients with T2D (352). The magnitude of BP reduction was similar to that observed in our study. There was also a statistically significant moderate correlation between ambulatory systolic BP reduction and LVM regression.

Interestingly, we also observed that there was a significant drop in nocturnal SBP rather than daytime SBP. This suggests dapagliflozin might result in at least partial restoration of circadian BP rhythm. The loss of nocturnal decline in BP has been established as an important marker for CV risk, independent of overall BP during a 24 hour period (354). The absence of the usual nocturnal fall in BP is associated with increased LVM (187). The extent of BP variability is independently associated with the rates of CV events and the extent of target organ damage (including LVH) (189)

Recently, a clinical case study examined dapagliflozin in patients with T2D who exhibited non-dipper type BP defined as a sleep-time mean SBP greater than 90% of awake time mean (356). Dapagliflozin significantly reduced BP and altered the circadian dipping pattern of BP, from a non-dipper type to a dipper type i.e. sleep mean SBP <90% of awake time mean. Another trial

using empagliflozin also revealed that the reduction in BP noted was greater during sleep-time, than during wake-time, in patients with hypertension and T2D (469).

Controlling BP is a standard approach to the management of LVH but this approach is only partially effective since 44% of all patients with type 2 diabetes are normotensive with LVH (17). This study included patients with BP within the normal range. Despite a normal "BP", normotensive LVH is just as risky as hypertensive LVH (70). LVH regression has been shown to be an effective way to reduce the incidence of all major CV events including specifically sudden deaths, heart failure hospitalisations, new onset atrial fibrillation and strokes independent of BP changes (289, 290, 293, 470-474). The LIFE study has conclusively shown that LVH regression *per se* reduces future CV events irrespective of BP changes (475, 476). There are other possible explanations for the reduction of LVM seen with dapagliflozin which include weight, preload (reduce ventricular loading conditions) and insulin resistance. Indeed, dapagliflozin had a greater effect on LVM in those with a blood pressure below the median.

4.1.2 Effect on Obesity Parameters

Obesity is a separate albeit related factor mediating LVH. We observed a correlation with LVM and BMI reduction and therefore a second plausible mechanism for LVH regression seen in this study may be dapagliflozin induced reduction in body weight. (231, 477). As discussed earlier obese subjects have an expanded central blood volume and increased peripheral resistance resulting in altered loading conditions that can directly lead to LVH (197). SGLT2 inhibitors have consistently been shown to lead to weight reduction of 2-3kg which is a consistent finding across the class of medications (352). The weight loss however does appear to plateau after 3-6 months (399). This mirrors the results of our study which showed that dapagliflozin significantly reduced weight on average by 4kg and the weight loss was most significant in the first 4-6 months of therapy. The effect of dapagliflozin was also greater in people with baseline weight above the median.

Potentially even more significant than simply just the weight reduction seen with dapagliflozin is that dapagliflozin resulted in a mean reduction in both VAT and SCAT. This is consistent with the results of other body composition studies with SGLT2 inhibitors (403-405). We observed a

significant correlation between LVM regression and VAT and SCAT suggesting this adipose tissue reduction was implicated in the LVM regression.

Both VAT and SCAT have been correlated with metabolic risk factors. In particular visceral fat is well recognised to be associated with an increased risk of T2D, CV complications and overall mortality and associated with insulin resistance, inflammation and oxidative stress (403-405, 478). Visceral adiposity is believed to be associated with these adverse outcomes primarily because of abnormal adipocyte biology and the release of biologically active substances including pro-inflammatory cytokines. Interestingly, the effect of dapagliflozin on LVM was concentrated in the subgroup with VAT volumes above the median. In the primary analysis dapagliflozin resulted in no change in LVMI to BSA due to the associated weight loss but in this subgroup there was a trend reduction in LVMI to BSA. This along with the strong correlation between LVM reduction and VAT reduction suggest these people have most to gain from dapagliflozin therapy and that VAT reduction is strongly implicated in the benefits in cardiac remodelling.

We explored if dapagliflozin reduced leptin, which as discussed earlier may have a pathophysiological role in sodium regulation as well as cardiac inflammation, fibrosis and remodelling (479). We did observe a reduction in leptin in the dapagliflozin arm, but this was not significant although this study was not powered for this outcome. We did not measure the anti-inflammatory adipokine adiponectin but dapagliflozin has been shown to significantly increase adiponectin concentrations in other studies when compared with other non SGLT2 oral hypoglycaemic treatment (411).

Although we did not see a significant reduction in leptin and did not assess adiponectin we did observe a reduction in hsCRP which might be secondary to an improvement in adipocyte function, and may contributed to the observed reductions in HbA1c and HOMA-IR (insulin resistance) levels.

Finally, epicardial adipose tissue is a reflection of visceral adiposity and has been implicated in the development of heart failure (480). Epicardial adipose tissue thickness is significantly higher in patients with type 2 DM (480). We did not specifically measure epicardial adipose tissue but

dapagliflozin has been shown to decrease epicardial adipose tissue volume whilst reducing other markers of inflammation such as TNF-α levels which is not surprising given the results seen in this study (439).

In summary as proposed in our hypothesis dapagliflozin therapy may have the potential to modify both the direct and indirect effects of obesity on the myocardium. At present though it is difficult to conclude whether the effect of SGLT2 inhibitors on adipose tissue inflammation is secondary to fat mass loss or a direct effect on adipose tissue function.

4.1.3 Effect on Preload

Dapagliflozin is a potent, competitive, reversible, highly selective and orally active inhibitor of SGLT2 receptors in the proximal renal tubule (327). As a consequence, dapagliflozin results in a dose dependant increase in urinary glucose excretion accompanied by an osmotic diuresis and natriuresis. This reduction in preload will improve the ventricular loading conditions reducing LV wall stress and contribute to dapagliflozin's ability to regress LVM in patients with LVH. We did not see any change in LV volumes, but baseline measurements were normal which may have made any changes difficult to detect. We also did not see any significant reduction in NT-proBNP which one might expect to see with reduced preload and myocardial stretch. However, dapagliflozin resulted in significant weight loss in the trial which is known to result in increased natriuretic peptide concentrations which may have counteracted any reduction with reduced preload (481).
We did see a significant increase in haematocrit in the dapagliflozin arm which may have been secondary to haemoconcentration as result of a reduction in plasma volume.
An alternative explanation may be an acceleration in haemoglobin production secondary to SGLT2 therapy. Inhibition of SGLT-2 transporters reduces oxygen demand in the renal proximal convoluted tubules thereby improving overall renal cortical ischemia and erythropoietin production by interstitial fibroblasts (482). This is important as anemia has been shown to increase morbidity and mortality of patients with HF.(483) Indeed, mediation analysis showed changes in Haemoglobin and haematocrit were the most important mediators of reduced CV death in the EMPA-REG OUTCOME trial (388). This may be one of the many important differences between SGLT2 inhibitors and classic diuretics. Another difference is SGLT2 inhibitors exert their effect in the proximal tubule of the kidney. SGLT 2 inhibition therefore results in an increased delivery

of sodium and chloride to the macula densa in the loop of henle downstream which may also limit the activation of the RAAS and SNS both of which can have an adverse effect of CV remodelling and subsequent CV outcomes. Indeed, we did not observe any significant increase in heart rate during ambulatory blood pressure recording suggesting a lack of compensatory sympathetic activation. Their effect in the proximal tubule is also associated with changes in adenosine bioactivity in the afferent renal arteriole reducing intraglomerular hypertension as discussed earlier. (394). We did not measure albuminuria but studies have shown that SGLT2 inhibitors are associated with reduced progression of albuminuria and slower decline in renal function when compared with placebo (395). The maintenance of total body salt and water homeostasis without the activation of the SNS and the inflammation associated with diabetic nephropathy may help reduce adverse LV remodelling as seen in this study with LVM regression. Hyperinsulinaemia can also cause renal sodium retention, and as we will discuss below dapagliflozin also reduced insulin and insulin resistance potentially further contributing to the beneficial effects of dapagliflozin on cardiac preload and LV remodelling (484).

4.1.4 Effect on Insulin Resistance

As discussed at length earlier insulin resistance is heavily linked to LVH and there are many proposed reasons for this. Hyperinsulinemia and insulin resistance is associated with alterations of myocardial metabolism leading to increased myocardial free fatty acids oxidation resulting in lipotoxicity and predisposition to cardiac hypertrophy and dysfunction (198, 485).
Hyperinsulinaemia and hyperglycaemia results in the formation of AGEs. AGEs contribute to increased connective tissue crosslinking, fibrosis, cardiac stiffness and therefore LVH and impaired diastolic relaxation (242). They are also involved in the production of ROS further contributing to the development of oxidative stress and subsequent inflammation and fibrosis noted in diabetic cardiomyopathy (242). Insulin resistance may promote myocardial hypertrophy and fibrosis through several signalling pathways, including Akt, transforming growth factor, and peroxisome proliferator-activated receptor (486).

We observed that dapagliflozin treatment resulted in a significant reduction in fasting glucose, fasting insulin and glycated haemoglobin. Due to time and money constraints we did not perform a hyperinsulinaemic euglycaemic clamp, the “gold standard” for the measurement of

insulin sensitivity but we did see that dapagliflozin resulted in a significant reduction in HOMA-IR an indices for insulin resistance/sensitivity. We did not observe a reduction in oxidative stress or fibrosis but did see a reduction in inflammation which may be secondary to VAT and insulin resistance reduction contributing to the beneficial effects on cardiac remodelling seen in this study.

4.1.5 Direct Cardiac Effects

Whilst dapagliflozin and other SGLT2 inhibitors can alter ventricular loading by way of blood pressure reduction, weight loss and diuresis as discussed above resulting in LVM regression, SGLT 2 inhibitors may also offer novel pathways in improving HF outcomes as well. In addition to their modest effect on ventricular loading by way of blood pressure reduction and increased diuresis, they may also have beneficial effects on myocardial bioenergetics, ion exchange, necrosis and fibrosis pathways as well as other metabolic and biochemical effects (455, 482). These novel molecular effects may also contribute to the rapid and striking improvements in HF-outcomes seen in the large SGLT2-inhibitor CV outcome trials noting the early divergence in survival curves.
In this study we attempted to explore some of these novel mechanisms however we did not observe any significant change in biomarkers of fibrosis or oxidative stress. We did, however, see a significant reduction in hsCRP which has been seen before in studies with dapagliflozin (436, 437). Chronic low-grade inflammation is recognised as a key feature associated with T2D and its complications including diabetic cardiomyopathy. The mechanisms by which dapagliflozin reduces inflammation are numerous and not mutually exclusive. As discussed above we suggest that as fat is more than just a simple energy storage compartment and can modulate inflammatory processes. The reduction in weight and total fat mass including visceral and subcutaneous adipose tissue along with improvement in insulin resistance leads to a less inflammatory state as observed with the reduction in hsCRP. This may well have also contributed to the positive effects on LVM.

4.2 Prognostic Benefits of Regressing LVH

As discussed at length earlier the regression of LVM is a very important finding as regressing LVH improves CV morbidity and mortality irrespective of BP changes. Numerous studies have consistently demonstrated this finding. The LIFE trial remains one of the largest trials to demonstrate that LVH regression is associated with a better prognosis even after accounting for

treatment induced BP reduction (296). Sub studies have shown LVM regression to be associated with significantly reduced risks for stroke, CVD and all-cause mortality independently of BP reduction (487). Other cohort sub studies on the LIFE trial, regression of LVH has been shown to be associated with reduced risk of new-onset heart failure, hospitilisation and overall mortality which is particularly relevant when considering the results of the SGLT2 outcome trials (293, 488). It is important to note that it was observed that LVM regression was less in patients with diabetes than in those without (299, 489). Therefore, the LIFE study clearly demonstrates the importance of regression of LVH, but it also reminds us of the importance of finding other ways to regress LVH in patients with T2D.

4.3 Other Studies Regressing Left Ventricular Hypertrophy Using SGLT2 Inhibitors

To the best of our knowledge, this is the first randomized controlled trial investigating the effect of dapagliflozin on LVH in people with T2D. Our findings are consistent with experimental mice and rat studies showing that SGLT2 inhibitors improve cardiac histopathologic changes in diabetic cardiomyopathy models. Kusaka et al demonstrated that 10 weeks empagliflozin treatment in genetic prediabetes/metabolic syndrome rats significantly reduced LV weight, cardiomyocyte size, cardiac interstitial fibrosis and cardiac interstitial macrophage infiltration (463).

With regard to clinical studies, the EMPA-HEART trial showed that empagliflozin promotes reverse LV remodelling in patients with diabetes (490). In total 97 patients with T2D with established CAD were randomised to empagliflozin or placebo for 6 months. Patients received a baseline cardiac MRI, with repeat cardiac MRI evaluation at 6 months. Empagliflozin resulted in a significant reduction in LVMI to BSA (-2.6 vs -0.01 g/m^2, p =0.01.).

Taking this all together would suggest SGLT2 inhibitors regress LVM. The importance of SGLT2 inhibition in diabetic patients with LVH has also been highlighted by a recent subgroup analysis of the EMPA-REG OUTCOME trial which demonstrated that the reduction of CV death, MI and stroke was greater in patients with LVH than in those without LVH (491). Furthermore, in this study LVH regression was greater in those with higher baseline LVM, which suggests that dapagliflozin has a greater effect in this higher risk subgroup.

4.4 Effects of Dapagliflozin on other Cardiac MRI Parameters

Over the course of the study dapagliflozin resulted in no other significant changes in other parameters measure on CMR, namely EF, EDV, ESV, SV and CO. This was consistent with the findings in the EMPA-HEART trial. It was also consistent with the results of the REFORM trial which was also performed in our department (492). It aimed to assess the effects of dapagliflozin in a heart failure population on left ventricular remodelling. There was no difference in the primary endpoints of LVEDV or LVESV. The neutral effect of dapagliflozin on LV volumes though is not entirely surprising. This trial and EMPA-HEART included patients without heart failure with normal LV volumes and therefore further improvements in remodelling would have been difficult in these circumstances. In the REFORM trial most of the patients were already on prognostic HF therapy and LV volumes at baseline were only mildly increased. In addition, during REFORM in the dapagliflozin arm the participants diuretics had to be significantly reduced to avoid hypovolaemia which may contributed to the neutral results.

Whilst it was not significant there was a reduction in left atrial volume in the dapagliflozin arm and trend reduction in left atrial area in the dapagliflozin arm in the per-protocol analysis. There was strong correlation between baseline LVM, and baseline left atrial area which is understandable as patients with higher LVM will have higher left ventricular end-diastolic pressures. The trend reduction in left atrial area was greater in the subgroup with LVMI above the median. This still did not reach significance but given this was a subgroup analysis the numbers were small, and the study was not powered for this endpoint. Left atrial enlargement is important because it has been found to independently predict the development of heart failure, atrial fibrillation and other CV events (146-148). Therefore, these results imply that dapagliflozin may prevent future atrial fibrillation as both left atrial area and LVH predict future atrial fibrillation which can exacerbate any cardiac events, however at present this is only speculative.

4.5 Effects of Dapagliflozin on Echocardiographic Parameters

Over the course of the study there were no significant changes in any of the diastolic function parameters in either the ITT or per protocol analysis. However, dapagliflozin has improved diastolic function in other studies albeit in mice and rat models. Eight weeks of treatment with

dapagliflozin in genetic diabetic mice improved EF and fractional shortening (449). Dapagliflozin also improved diastolic function in a diabetic non-obese mouse model (459). Dapagliflozin improved the E/A (early/late diastolic) ratio, isovolumetric relaxation time (IVRT), deceleration time (DT) and end diastolic wall thickness (EDWT).

Verma et al in an uncontrolled case series demonstrated that empagliflozin may improve diastolic function (464). Empagliflozin therapy appeared to improve diastolic function as assessed by the early lateral annular tissue Doppler velocity (8.5 (1.6) vs 9.6 (1.3) cm/s, p=0.002). These findings were however preliminary and only in a small number of patients. As discussed above, we did however note a reduction in left atrial volume and left atrial area which was more pronounced in participants with LVMI above the median. These changes were not significant, but this study only included small numbers and was not powered for this endpoint. It's important to note though as the left atrium is exposed to left ventricular pressure during diastole. It is therefore susceptible to remodelling through increasing pressure and volume (493). Left atrial size is therefore considered as a marker of left ventricular diastolic function (468).
However, volume is an insensitive biomarker of the early phases of left ventricular diastolic dysfunction. LA phasic function can be assessed both by volumetric analysis, using 3-dimensional echocardiography, and by strain/strain-rate analysis, using speckle-tracking echocardiography (494). None of these methods were performed in this study but it is becoming increasingly recognised that the measurement of LA function improves not just the diagnostic accuracy but the prognostic value of both left ventricular diastolic function and HFpEF algorithms.

Dapagliflozin in our study did result in a significant improvement in global longitudinal strain. It must be stressed that these results should be interpreted with caution as only small numbers were included in the analysis. However, they do warrant further thought and research. Subclinical LV dysfunction is highly prevalent in type 2 diabetes patients (132). Speckle tracking is a validated non-invasive method for evaluation of ventricular systolic function (495, 496). Among patients with T2D who have no previous cardiac events, valve disease or symptoms as many as 30-50% of these patients have abnormal longitudinal strain despite documented normal LVEF (497, 498). Our findings may suggest that dapagliflozin does improve subclinical LV systolic function not detected by LVEF. We did note an improvement in LVEF in the dapagliflozin arm, but this was not significant. The EMPAHEART trial also noted a trend towards improvement in LVEF with an

improvement of 2.2% in the empagliflozin arm vs -0.01% in the placebo arm (p=0.07) (490). Sakai et al have also demonstrated SGLT2 inhibitors improve both diastolic function and GLS in patients with T2D and HFpEF (499). Regression of LVM and reduction in adverse remodelling may contribute to this improvement in subclinical LV function. Another mechanism suggested is that of enhanced myocardial energetics. The healthy heart can freely switch between free fatty acids(FFA), glucose, ketones and lactate as fuel depending on workload, and substrate availability (500). In the insulin-resistant state of T2D, glucose is unable to enter the cardiomyocyte forcing it to utilize FFA which is less oxygen efficient. Ketone bodies are more oxygen efficient but less readily available (501). We did not measure ketone bodies but our colleagues did in REFORM who demonstrated that dapagliflozin does increase BHB (492). Studies in patients with HF show BHB increases cardiac output, LVEF and myocardial oxygen consumption (502). One can speculate that an increased supply of highly efficient fuel and improved oxygen delivery due to the increased haemoglobin and haematocrit results in improved cardiac function.

Overall at present whether SGLT2 inhibitors leads to a significant improvement in cardiac function though remains in doubt. However, further evaluation of SGLT2 inhibitors on left atrial function and GLS would be interesting and highly relevant to clinical practice as treatments for HFpEF remained limited. It may also shed light on which patients might benefit most from SLT2 inhibitor therapy.

4.6 Side effects and Tolerance of Dapagliflozin

Overall dapagliflozin was well tolerated in this study. One patient did have to withdraw due to hyponatraemia (as per the protocol withdrawal stipulations) in the dapagliflozin arm. They were however also on Bendroflumethiazide which may well have contributed to the hyponatraemia.
The incidence of common side effects reported with SGLT2 inhibitors were not excessive but there were significantly more episodes of reported hypoglycaemia and thrush in the dapagliflozin arm. Interestingly there was significantly more urinary tract infections in the placebo arm although one participant in the placebo arm accounted for three of the reported urinary tract infections in this arm. Importantly there was no reported episodes of diabetic ketoacidosis/bone fracture/thromboembolism or CVA events.

4.7 Clinical Relevance

In this study we have shown that dapagliflozin regresses LVH in patients with T2D and controlled BP. This study is highly relevant and topical following the observed CV benefits seen with SGLT2 inhibitors in the recent large CV outcome trials including most recently the DAPA-HF trial. These outcome trials were particularly noteworthy as previously trials such as the three large randomized controlled trials (RCTs) ADVANCE, ACCORD and VADT failed to demonstrate any significant effect on macrovascular events of more intensive glycaemic control in patients with longstanding T2D when compared with standard medical care (414-416). This suggests the mechanisms for the benefits of SGLT2 inhibitors are independent of glucose lowering. This is likely to be the case with the recent release of the top line results of the Dapa-HF trial that reported positive results showing that dapagliflozin met the primary composite endpoint with a statistically significant and clinically-meaningful reduction of cardiovascular death or the worsening of heart failure in patients with or without T2D (351).

Whilst this study was too small to examine CV outcomes over time if we compare the results with the LIFE study where regressing LVH was associated with improved CV outcomes they are not too dissimilar. In the Echo sub-study of LIFE the difference in LVM between the two active treatments was only 3% . In this study the difference between dapagliflozin and placebo was around 2.5% for LVM. In LIFE there was a 14% reduction in CV events and a 25% reduction in strokes (487). Hence this study could be clinically very relevant if we extrapolate the LIFE improvement in CV outcomes with small reductions in LVM to our study. Furthermore, this study was only conducted over 12 months. Therefore, one could possibly expect further reductions in LVM over a longer duration of time. As discussed previously at length a reduction in LVM is an important observation because it is an independent predictor of CV events.

The observed reduction in SBP, increased haematocrit and reduced measures of obesity such as body weight, SCAT and VAT and reduced insulin resistance and markers of inflammation is also important. This is because it demonstrates that dapagliflozin appears to reduce all four of the known causes of LVH i.e. BP, preload, obesity and glycaemia/insulin resistance which is unique as all other antidiabetic and antihypertensive drugs only reduce one or two.

LVH is a good surrogate marker of CV outcome, and this mechanistic study suggests a potential mechanism particularly for the HF benefits, noted with SGLT2 inhibitors in the three large CV outcome trials and the recent DAPA-HF trial.

4.8 Limitations

This was a single centre study with relatively small number of patients. However, this trial is the first prospective, adequately powered RCT conducted to date, investigating the efficacy of dapagliflozin to regress LVH. Secondly, the study was statistically powered only for a single outcome and not statistically powered to detect changes in other secondary end points. Therefore, inferential between group comparisons for these secondary endpoints are likely to be exploratory rather than definitive.

As discussed, LVH is characterized by reactive fibrosis, with disproportionate accumulation of collagen in the heart. This contributes to the development of diastolic dysfunction, heart failure and sudden cardiac death (168). In therefore study we did not use contrast to perform late gadolinium imaging to assess myocardial scar and fibrosis. In our experience patients only return for second MRI scans if the scan time is no more than around 45 minutes. Given this time constraint we thought it to be more novel to do adiposity measures rather than more LV parameters. Whilst we did see a significant reduction in HOMA-IR a marker of insulin resistance, due to time and money constraints we did not perform a hyperinsulinaemic euglycaemic glamp which is the "gold standard" for the measurement of insulin sensitivity. Therefore, further studies would be needed prior to making any definitive conclusions.

Finally, although there were no statistically significant differences between the two groups, because of the relatively small sample size, we cannot exclude the possibility that some subtle baseline and demographic differences between two groups, might have collectively contributed to our results.

4.9 Future Research

4.9.1 Patients with Heart Failure

This study was performed in patients with diabetes without heart failure. It was a mechanistic study to evaluate the possible mechanisms behind the significant benefits primarily in the reduction in HHF seen with empagliflozin in EMPA-REG trial. Most patients in the SGTL2 outcome trials did not have heart failure. Therefore, a huge question is whether SGLT2 inhibitors might also benefit patients with established heart failure and given that the mechanisms appear independent of glucose lowering, whether the benefits extend to patients without type 2 diabetes. The recent

publication of the Dapagliflozin And Prevention of Adverse-outcomes in Heart Failure trial has shown that dapagliflozin reduces death and hospitalisation, and improves health-related quality of life, in patients with established heart failure and reduced ejection fraction, with and without diabetes. The clinical implications of these findings are potentially huge as few drugs achieve these results in heart failure and dapagliflozin appears to do this even when added to excellent standard therapy (503). The next logical step would be to establish if SGLT2 inhibitors can be used in hospitalised patients with acute decompensation.

4.9.2 Subclinical Left Ventricular Dysfunction

This study suggests dapagliflozin may have benefits on left ventricular function not detected by LVEF. HFpEF, is a common, disease sorely in need of effective pharmacotherapies and carrying on with the theme that these agents may actually be cardiovascular drugs that happen to lower blood glucose, a number of placebo controlled trials are looking into the effect of SGLT2 inhibitors on HHF in people with HFpEF without diabetes. The EMPEROR-PRESERVED and the DELIVER trial are assessing empagliflozin and dapagliflozin respectively in this cohort with both due to complete by mid 2021(504, 505) .

4.9.3 Myocardial Fibrosis

Left ventricular hypertrophy is associated with diffuse reactive fibrosis characterized by accumulation of collagen (168). This diffuse myocardial fibrosis is the core of LV remodeling and therefore the interstitium of the myocardium has become the subject of intense research interest but previously the diffuse nature of the fibrosis has made it impossible to detect such interstitial fibrosis by standard T1 weighted imaging (506). Advances in cardiac magnetic resonance imaging recently have made it possible to quantify the T1 properties (T1 relaxation times) of the heart and T1 maps can detect even relatively small variations of T1 within the heart muscle to demonstrate myocardial tissue pathology (506). T1 mapping can be used to calculate extracellular volume which quantifies the relative expansion of the extracellular matrix as a result of diffuse reactive fibrosis (506). Given these imaging developments and the finding that dapagliflozin regresses LVH it would be fascinating to see the if SGLT2 inhibition reduces diffuse myocardial fibrosis as well as LVM.

4.9.4 Left Atrial Function

As discussed above left atrial size is a marker of diastolic function. The left atrial size reduction seen in this study albeit not significant in this study suggests that dapagliflozin may improve diastolic function and reduction in left atrial size may have benefits in preventing AF developing. Left atrial function is more sensitive marker of diastolic function and studies evaluating the effect of SGLT2 inhibitors on left atrial function my provide further insight in this area which would be relevant to clinical practice given the paucity of HFpEF treatments.
Also given the close correlation of atrial dilatation and the development of atrial fibrillation, the occurrence of atrial fibrillation and recurrence of atrial fibrillation would be interesting endpoints of future dapagliflozin outcome trials.

4.10 Final Summary/Take Home Message

In conclusion, this study has shown, for the first time in a randomized controlled trial that dapagliflozin treatment significantly reduces LVM compared to placebo in people with T2D, LVH and controlled blood pressure. This is consistent with the results seen with empagliflozin in EMPA-HEART and these independent reports provide excellent validation for both studies (465). Three large CV outcome have demonstrated the benefit of SGLT2 inhibitors on HHF. By contrast to the clear benefit on HHF, none of the 3 trials showed a convincing effect of SGLT2 inhibition on atherothrombotic events. A recent meta-analysis of the 3 trials confirmed the robust effect of SGLT2 inhibitors in reducing HHF/CV death by 23% (507).

This mechanistic study showing the regression of LVM suggests dapagliflozin can initiate reverse remodelling and changes in left ventricular structure which maybe a potential mechanism for the predominant effect on HF benefits, noted with SGLT2 inhibitors in the three large CV outcome trials including the recent DAPA-HF study.

4.11 Prizes, Publications and Poster Presentations

4.11.1 Prizes

Winner of Fitzgerald Peel Prize for best oral presentation at the Scottish Society of Physicians 61st Annual Meeting 2019. Abstract 'Dapagliflozin Regresses Left Ventricular Mass in Patients with Type 2 Diabetes and Left Ventricular Hypertrophy

4.11.2 Publications

2017 **Alexander Brown,** Chim Lang, Rory McCrimmon, Allan Struthers.
Does Dapagliflozin regress left ventricular hypertrophy in patients with type 2 diabetes? A prospective, double blind, randomised, placebo-controlled study. *BMC Cardiovascular Disorders, vol 17, 229, pp1-12.*

2019 **Alex Brown,** Stephen Gandy, Rory McCrimmon, Allan Struthers, Chim Lang
Abstract 10643: A Randomised Controlled Trial of Dapagliflozin on Left Ventricular Hypertrophy in Patients With Type Two Diabetes. The DAPA-LVH Trial. *Circulation 2019;140:A10643*

4.11.3 Poster Presentations

American Heart Association

2019 **Alex Brown,** Stephen Gandy, Rory McCrimmon, Allan Struthers, Chim Lang.
A Randomised Controlled Trial of Dapagliflozin on Left Ventricular Hypertrophy in Patients with Type Two Diabetes. The DAPA-LVH Trial
(In top ten most viewed posters at the conference)

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4.13 Appendices

4.13.1 Consent Form

PATIENT INFORMED CONSENT FORM

DAPA-LVH STUDY - Does Dapaglifozin Regress Left Ventricular Hypertrophy In Patients With Type 2 Diabetes?

		Initials
Participant Number _____ Participant Initials____		
I have read and understood the Participant Information Sheet for DAPA-LVH Study, Version 7 28-06-18	Yes☐No☐	☐
I have spoken to: _____		
I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	Yes☐No☐	☐
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without any medical care or legal rights being affected.	Yes☐No☐	☐
I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the co-Sponsor, University of Dundee and NHS Tayside or on their behalf by a third party where it is relevant to my taking part in this research. I give	Yes☐No☐	☐

permission for these individuals to have access to my records.

I agree to my GP being informed of my participation in this study.	Yes☐No☐	☐
I understand, and I agree that my identifiable routine blood tests and MRI scan result will be stored within the NHS clinical system and will be available to doctors looking after me in the future.	Yes☐No☐	☐
I agree to be informed of any significant clinical finding found during my participation in the research project and agree that members of the research team can contact both me and my GP and inform any referral specialist required to carry out further investigations.	Yes☐No☐	☐
I agree that If I withdraw or I am withdrawn from the study that data already collected can be retained and included in the data analysis.	Yes☐No☐	☐
I understand and agree that the research blood samples that I provide will be gifted by myself, transferred to specialist laboratories in the School of Medicine, University of Dundee and analysed by members of the study team or stored (link-anonymised) for up to 15 years. This can be used for future, as yet unspecified, medical research, which may include genetic research. This research will be subject to proper scientific and ethical review.	Yes☐No☐	☐
I agree to take part in the above study.	Yes☐No☐	☐

This research is approved by East of Scotland Research Ethics Committee REC2

_____	_____	_____
Name of participant	Date	Signature

_____	_____	_____
Name of person taking consent	Date	Signature

1 copy to be given to the patient; 1 copy to be filed in the patient's hospital notes and the original to be kept in the ISF

4.13.2 Patient Information Sheet

DAPA-LVH STUDY - Does Dapaglifozin Regress Left Ventricular Hypertrophy In Patients With Type 2 Diabetes?

PARTICIPANT INFORMATION SHEET

We would like to invite you to participate in a research project which is being carried out by Professor Chim Lang and [Dr Alex Brown] (University of Dundee).

Before you decide whether or not to participate, we need to be sure that you understand firstly why we are doing the study and secondly, what it would involve if you agreed to take part. We are therefore providing you with the following information. Please take time to read it carefully and be sure to ask any questions you have and discuss it with others if you want. We will do our best to explain the study and provide any further information you may ask for now or later. You do not have to make an immediate decision.

Why are we doing this study?

People with diabetes are at increased risk of developing thickening of the heart muscle on the left side of the heart. This is known as Left Ventricular Hypertrophy (LVH). People with LVH are 2-5 times more likely to have a heart attack or stroke. LVH can often be caused by high blood pressure, however diabetes and being overweight may also contribute to its presence in people with normal blood pressure.

Dapaglifozin, a medicine used to treat diabetes, can improve blood sugar levels, reduce blood pressure and cause weight loss and may therefore reduce the thickening of the heart muscle. This might be a new way to reduce the risk of heart attack or stroke in people with diabetes who also have LVH.

This study will investigate the ability of Dapaglifozin to reduce the thickening of the heart muscle in people with diabetes and LVH and will performed as part of a MD thesis for Dr Alex Brown. We aim to recruit 64 people between the ages of 18 and 80 years who have diabetes and have LVH but have normal blood pressure.

To decide if you are able to take part in the study, the thickness of the heart muscle will be measured at the start of the study by an ultrasound scan. Blood pressure will also be measured over a 24 hour period. Only people who have a thickened heart muscle, LVH, and normal blood pressure will be asked to continue in the study. If you are suitable to continue in the study your LVH will be measured more accurately with a Magnetic Resonance Imaging (MRI) scan.

Participants will be randomly allocated to receive either Dapaglifozin or a dummy medication (placebo). Participants will receive one year of treatment and we will compare if there is a difference between normal treatment and addition of Dapaglifozin at the end of that period. All participants will continue to take all currently prescribed medication, as well as the study drug. However, if you are on insulin, we will reduce your total insulin dose by 10% on the day we start you on the study medication (Visit 2).

Why have I been contacted?

This study is looking at the effects of Dapaglifozin in people with type 2 diabetes. We have contacted you because you have diabetes and you may therefore be suitable to take part.

The study is not suitable for women who are pregnant, breast feeding or who are trying to become pregnant.

Do I have to take part?

It is up to you to decide. Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without having to give a reason and without this affecting your future medical care or your relationship with medical or nursing staff looking after you. If you, [Dr Alex Brown] or one of your clinicians decides you should withdraw from the study, we would like your permission to retain and analyse the data already collected.

What will happen to me if I take part?

This study takes place at Ninewells Hospital, Dundee. You will be in the study for between 12 to 13 months, with 6-8 visits scheduled to take place at your convenience. We can pay for a taxi or other transport to get you to the hospital and back for each study visit if this would be helpful.

You will be given a tablet which contains the medication we are testing (called dapagliflozin) or a dummy tablet (called a placebo) and will be asked to take one tablet per day while you are in the study. Before you start your study tablets you will be asked to come for a screening visit to check you are eligible for the study.

Visit 1 - Screening
At the start of this visit the doctor or research nurse will discuss the study with you and ask you to sign a consent form stating that you have read this information sheet and that you agree to take part in the study.

The doctor will assess your suitability for the study, this will take about 1½ hours. At the screening visit the doctor will ask you about your medical history and what medications you take, and do a clinical examination to ensure you are suitable to take part in the study. The doctor or research nurse will check your blood pressure, pulse, temperature, height, weight, perform an electrocardiogram (ECG) and do some routine blood tests. The blood tests (safety bloods) will check your liver and kidney function, cholesterol level,confirm you are not anaemic and to check how well your diabetes is managed to be sure that it is safe for you to participate. The total volume of blood taken at this visit will be approximately 20ml (4 teaspoons full).

You will have an echocardiogram performed at this visit to measure the thickness of your heart muscle. Only people who have a thickened heart muscle, LVH, will be asked to continue in the study. Visit 1 is then complete.

Once we have reviewed your blood results and confirmed it is safe for you to proceed in the study, you will be contacted and asked to return for an MRI Scan (further details below) and your next study visit.

If anything is discovered in your screening visit that is of concern and prevents you from taking part we discuss this with you and, if necessary, will arrange the appropriate medical follow up for you. With your consent, we will also let your GP know.

If you have had a penetrative eye injury or exposure to metal fragments in your eye(s) that required medical attention, you will be advised that it is unsafe for you to continue further in this study as there is a risk that the magnetic field in the MRI scan could move the metal fragment which may cause harm to your eye. With your consent, we will write to your GP informing them of your MRI safety status, as this information may be of benefit for your future health care needs.

Contraceptive Advice
Anyone who is pregnant cannot take part in this study. If you are a woman of childbearing potential, this means you have not passed menopause (over the age of 45 and not had a period for more than 12 months) and have not been surgically

sterilised or had a hysterectomy (womb removed), bilateral salpingectomy (both tubes removed) or bilateral oophorectomy (both ovaries removed) you will need to use contraception during the period of the study.

These are effective types of contraception:

- Combined Oral Contraceptive Pill
- EVRA-osetrogen and progestogen: 'Transdermal Patch'
- Progestogen only pill: 'mini pill'
- Depoprovera injection (medroxyprogesterone acetate)
- Implanon Implant (Etonogestrel)
- Mirena Coil (Intra-Uterine System)
- IUD-copper containing intrauterine device
- Male condom

Male vasectomy is also a good form of contraception but only if the procedure has been checked afterwards by your doctor to make sure it has worked.

No contraception method is 100% reliable by itself. Even surgical sterilisation in men and women has been known to fail very occasionally. We advise using additional contraception from the start of the study.

Pregnancy test: all women of child bearing potential will have a pregnancy test to confirm they are not pregnant.

Baseline MRI Scan

You will be sent an appointment either by telephone call, letter or e-mail for your MRI scan. The MRI scan will take place at the MRI department of the Clinical Research Centre, Ninewells Hospital, Dundee (directions will be included). Where possible the appointment for your MRI scan will be on the same day as your visit 2 but it may require a separate visit.

Before your scan you will meet one of the research team who will check that you are eligible to have the scan and who will obtain your written consent. You will then be seen by the radiographer, the person taking your scan, and she/he will help you to complete a checklist about matters that might prevent you from having the scan.

If you are a woman of child bearing potential you will be asked to provide a urine specimen which you can either bring with you (a specimen bottle will be provided) or provide at the beginning of your MRI clinic visit. A pregnancy test will be performed to ensure your safety. A positive result will exclude you from having an MRI scan and continuing in the study.

If the radiology staff establish that it is safe to scan you, you will proceed with your MRI and will be asked to change into a gown for the scan. You will be asked to lie on the scanning table and will be moved into the center of the scanner (the scanner is shaped like a big doughnut). During the scan, which takes around 45 minutes, you will be able to speak to the radiographer. The scan will take pictures of your heart and abdomen. As the scan is noisy you will be wearing hearing protection. A specialist will

examine your scan at a later date for any signs of disease and will measure the thickness of your left heart muscle as well as the amount of fat in your abdomen.

This MRI visit should take no longer than 1½ hours.

Visit 2 - Baseline
This visit will take place anytime up to three weeks before or after the MRI scan but where possible will be done on the same day as the MRI scan. This visit will take about 1 hour plus the time taken for your MRI scan if this is being done on the same day.

You will be asked to fast for this visit so that we can measure your blood glucose and insulin levels accurately. You will be given instructions on how long you need to fast for before you come for your visit. You will still be allowed to drink water when fasting and during any visit. The study doctor will also advise you on when to take you usual medications, but it is important that you do not take your diabetes medications (including insulin if you take this) until AFTER your blood samples have been taken. Food and drink will then be provided so that you can take your diabetes medication safely.

Visit 2 will include the following investigations:

Vital signs
Checks of your blood pressure, pulse and temperature. Your waist and hips will also be measured.

Blood tests
Safety bloods: To check your liver and kidney function, cholesterol level, confirm you are not anaemic and to find out how well your diabetes is controlled.

Research bloods: will be taken and stored for analysis after the study has ended. The research bloods will look at markers of heart function and glucose and insulin levels.

Total blood taken will be approximately 40ml (2½tablespoons). Following completion of the study we may test for additional markers of interest on any left-over blood which will be stored anonymously in the secure Dundee University laboratory in the division of Cardiovascular and Diabetes Medicine for up to ten years. This may include genetic tests.

Pregnancy test
Women who are of child bearing potential will have a pregnancy test done at every visit. We will also give you pregnancy test kits to take home with you and ask you to do a pregnancy test every 4 weeks. The Researcher will phone you to see what the result of this home test is. If positive you will not be able to continue in the study and your study medication will be stopped.

24 hour blood pressure recording
You will be fitted with a blood pressure monitor which will record your blood pressure regularly for 24 hours. You will be given a pre-paid envelope to post it back to the study team. If you are unable to carry out 24 hour BP measurements, we would allow daytime (16 hour) measurements to avoid measurements at night.

Study medication

At the end of this visit you will be randomly allocated to receive either Dapagliflozin (10mg) or placebo. The tablets allocated to you are decided in a random way (a bit like tossing a coin) and neither you nor the research staff will know which tablet you are taking until after the study is completed. This ensures that the study results cannot be influenced by knowing whether you are receiving the medication or not.

You will then be given enough study drug to take once daily for four weeks.

Visits 3, 4 & 5

Visit 3 will occur approximately 4 weeks after visit 2, with visits 4 and 5, 4 months and 9 months after visit 2. Again you will be asked to fast before your visit. At these visits you will have the same assessments as were done at visit 2 except the 24 hour blood pressure recording and MRI scan. At the end of each visit you will receive a further supply of study medications to last you until the next visit.

Visit 6 – final visit

This is the final study visit and will occur approximately 12 months after visit 2. You will be asked to fast for this visit as well. At this visit you will have the same assessments as at visit 2 including a repeat echocardiogram and repeat MRI scan (within 3 weeks of the visit) and have the 24 hour blood pressure monitor fitted. All the assessments will be compared with the baseline assessments taken before you started on the study medication. With your permission we will also take an extra 20ml sample of blood for research purposes. This sample will be stored in the secure laboratory within the Division of Cardiovascular and Diabetes Medicine at Ninewells Hospital.

For all visits noted above the doctor will assess you for any side effects of the medication and will check your vital signs and do blood tests to assess if the Dapagliflozin has caused any problems.

Visit	**1** **Screening** **(before start)**	**2** **Baseline** **(start)**	**3** **4 weeks**	**4** **4 months**	**5** **9 months**	**6** **12 months**
Informed consent	√					
Medical history & medications	√	√	√	√	√	√
Physical examination	√					
Height & weight	√					
Blood pressure & pulse	√	√	√	√	√	√
Temperature	√					
ECG	√					
Echo heart scan	√					√
Blood samples	√	√	√	√	√	√
Pregnancy test (if applicable)	√	√	√	√	√	√
Waist & hip measurement		√	√	√	√	√
24 hour blood pressure		√				√
Heart & abdominal MRI scan		√				√
Adjustment of usual diabetes or blood pressure medication		√	√	√	√	
Medication supply		√	√	√	√	

What happens if I or my partner becomes pregnant?

If you become pregnant during the study, the study team will need to be informed. You will be withdrawn from the study, and will be asked to give consent to be followed-up until then end of the pregnancy and report the outcome including that of your infant.

If you are male and your partner becomes pregnant we request that you inform us and we will ask your partner's consent to follow them up to the end of their pregnancy and report the outcome including that of your infant.

What is the medication being tested?

The medication used in this study is called Dapagliflozin. It is used for the treatment of type 2 diabetes and has been licensed for that use in Europe since 2012. It works by lowering blood glucose (sugar) by increasing the amount of glucose removed by the kidneys.

Dapagliflozin is generally well tolerated, however, like most medicines, dapagliflozin occasionally causes side effects.

Among the known side effects of dapagliflozin are diabetic ketoacidosis (DKA) (a build-up of acid in the blood), hypoglycaemia (low blood sugar), urinary tract infection, genital tract infection and increased urine production. The most serious risks are also the rarest, DKA may affect between 1 in 1000 to 1 in 10,000 patients. To lower this small risk even further, we will give you information about the symptoms of DKA to look out for and what to do if you notice any symptoms. We will be monitoring for these symptoms and other features of DKA during every visit.

Major hypoglycaemia may affect between 1 in 200 to 1 in 250 patients. To address the risk of hypoglycaemia, participants taking insulin will be asked to reduce their insulin dose by 10% when they join the study. Their laboratory-based blood sugar levels (along with home-monitored levels) will then be monitored regularly by the study team, and the necessary dose adjustment will be done. We will be providing you with written information on the possible symptoms of hypoglycaemia and how to manage it.

Other common non serious side effects are: runny or stuffy nose; sore throat.

The complete range of reported side effects is set out in a Package Information Leaflet, a copy of which will be given to you at your screening visit for your information. This will be further discussed with you before you make a final decision about taking part in this study.

Will taking part in the study affect your usual care?

Your diabetes and blood pressure will be reviewed at every visit by the study team to aim to have your diabetes or blood pressure managed as per local clinical guidelines. If [Dr Alex Brown] thinks that you would benefit from having your usual medication altered they will first discuss this with you and then discuss this with your GP who may change or add to the medications you are currently prescribed. If your medications are changed and [Dr Alex Brown] thinks you should be reviewed before your next visit for the study or have any further assessments, such as blood tests, this will be discussed with you and a convenient time for you to come to Ninewells Hospital to have this review will be made.

What are the discomforts, risks and side effects of taking part?

The side effects of the dapagliflozin are discussed under the 'medication' section above.

Having blood tests taken can cause some mild bruising.

MRI scanning: This type of scan is very safe and does not use radiation. Some people may feel a bit closed in when being scanned but you will be in constant contact with the person performing the scan and you can come out at any time. The scanner is a bit noisy but you will be given ear protection which also plays music.

What are the benefits of taking part in the study?

You will be monitored closely during the study and will be seen by a doctor with a special interest in heart medicine (cardiology) at each of your study visits.

The assessments will give us information about the function of your heart, kidneys and liver. If any of these investigations, including information from the MRI scan of your heart, reveal any new abnormality we will first of all discuss this with you and then discuss this with your GP, hospital consultant or refer you to a specialist clinic (whichever seems most appropriate).

The study will not immediately benefit you, but if the results of the study are positive it may change the practice of managing people with diabetes and LVH. If so, you may gain eventually from our discovering a new treatment for your condition.

Will my GP know about this research project?

With your permission we will inform your GP of your participation, any clinical results, and of any new medical problem we find as a result of your participation in the study.

Will my taking part in this study be kept confidential?

All the information that is collected about you during the course of this study will be kept strictly confidential. There will be two sets of information obtained during the study. One set will be routine blood tests analysed by local NHS laboratories and MRI scan results and the other, the research data obtained from research blood samples and study procedures. The routine blood test and MRI scan results will be stored indefinitely using your name and unique hospital record number within the NHS clinical system and are available to specialist NHS doctors and GP for your future health care needs.

Your research data will be stored using a unique study code which is non-identifiable. All written information will be kept in a locked filing cabinet in a locked room. Any computer based data will be stored in a secure, password protected database in the Division of Cardiovascular and Diabetes Medicine, University of Dundee at Ninewells Hospital. Only individuals directly involved with the study will have access to this information. It is a requirement of the regulators that your records in this study, together with any other relevant medical records, be made available for scrutiny by appropriate monitors from University of Dundee, NHS Tayside, and the Regulatory Authorities. This procedure is routine and carried out by fully qualified staff, and data confidentiality is preserved.

At the end of the study the confidential records will be kept for 15 years and then destroyed. The confidential handling, processing, storage and disposal of data are in accordance with the Data Protection Act 1998.

The research blood tests will be analysed by our laboratory staff in the Division of Cardiovascular and Diabetes Medicine, University of Dundee at Ninewells Hospital. After the tests are complete, we will, with your permission, store the spare blood samples so that we can perform further tests on them in future.

Will I continue to receive the medication used in this study after it finishes?

Not usually. The study gives an indication of possible benefit from the medicines being tested and it may be some time before we are sure about how useful it actually is. Unless you need the medication for your diabetes, you would not receive the medication after the end of the study.

What will happen to the results?

The results will be examined by the researchers who have organised the study and a short report will be produced. You will not be identified in this report. The results will be shared with the funder for the study (AstraZeneca). The results will then be published in scientific journals. Again, you will not be identified in any journal articles. If you would like the results of the study please ask the research team.

Who is organising and funding this research?

The study has been organised by Professor Chim Lang and colleagues at the University of Dundee. This project is funded by AstraZeneca.

What are my rights?

If you have a complaint about your participation in the study you should first talk to a researcher involved in your care. You can ask to speak to a senior member of the research team or the Complaints Officer for NHS Tayside.

Complaints and Feedback Team
Ninewells Hospital
Dundee
DD1 9SY
Telephone: 0800 027 5507
Email: feedback.tayside@nhs.net

In the event that something goes wrong and you are harmed during the study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Dundee or NHS Tayside but you may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you (if appropriate.)

Who has reviewed the study?

The East of Scotland Research Ethics Service REC 2 which has responsibility for scrutinising proposals for medical research on humans, has examined the proposal and has raised no objections from the point of view of medical ethics.

For further information contact:

Principal Investigator: Dr Alex Brown a.y.brown@dundee.ac.uk or 01382 383391

If during the study you become unwell or are concerned, as well as the usual services provided by the NHS such as NHS24 (Tel: 111), you can also contact the study team during normal working hours on (01382) 383086 if you are unwell and need urgent advice or assistance do not delay in seeking further advice or treatment as usual through the NHS services.

Thank you for reading this information sheet and considering taking part in this study. If you would like more information or want to ask questions about the study please contact the study team on the number/addresses above.

4.13.3 GP Letter

☏ 01382 383391
Email address: a.y.brown@dundee.ac.uk

DAPA-LVH STUDY - Does Dapaglifozin Regress Left Ventricular Hypertrophy In Patients With Type 2 Diabetes?

[xx/xx/xxxx]

Dear Dr

Your patient has kindly consented to join the DAPA-LVH trial.

Patient details:

Name:

CHI:

Address:

DAPA-LVH is a single centre, parallel-group randomised controlled trial evaluating dapagliflozin in 64 people with type 2 diabetes and left ventricular hypertrophy (LVH). The trial is recruiting patients from Tayside. Male and female patients with type 2 diabetes and LVH are eligible for participation. Patients will have an echo to assess if they have LVH prior to starting in the study. The trial will assess the impact of dapagliflozin on LV mass.

Participants will receive a total of 12 months of either dapagliflozin 10mg daily or placebo, with LV mass measured by MRI at 0 and 12 months. As dapagliflozin may cause hypoglycaemia we will ask patients who are taking insulin to reduce their dose by 10% at the start of the study. During the 12 months patients are in the study we will be assessing the diabetes management and blood pressure regularly and aiming for a HbA1c target of <53mmol/mol and blood pressure <140/90 mmHg. I may therefor contact you to discuss the management of your patient’s diabetes and/or blood pressure.

Participants currently taking loop diuretics or currently receiving long term (>30 consecutive days) treatment with an oral steroid will be excluded from the study. Participants who are prescribed loop diuretics or steroids during the trial period will have their study medication permanently stopped but will remain in the study. I would therefore be grateful if you could inform me as soon as possible if patients require to be commenced on loop diuretics or steroids.

If any significant clinical findings are found during your patient's participation in this study you will be informed of the results and any relevant corrective action implemented.

If you have any questions please do not hesitate to contact me.

Thank you for your assistance.

With kind regards,

Dr Alex Brown

Clinical Research Fellow
Mailbox 2 Department of Cardiovascular and Diabetes Medicine Medical Research Institute
Level 7
Ninewells Hospital & Medical School
Dundee
DD1 9SY

YOUR PATIENT HAS BEEN UNABLE TO ENTER INTO THE STUDY (SCREEN FAIL, see below)	☐
YOUR PATIENT HAS BEEN ENTERED INTO THE STUDY	☐
NOTABLE FINDINGS AT SCREENING VISIT	

4.13.4 Case Report Form

DAPA-LVH

Case Report Form

Does Dapagliflozin Regress Left Ventricular Hypertrophy In Patients With Type 2 Diabetes?

Participant Initials		**Date of Birth**	
Participant ID		**Randomisation ID**	

Visit	Week	Date	Time	Taxi Required?	Taxi Booked?	Comment
Visit 1 ***(Screening)***	- 4					
Visit 2 (a) ***(Randomisation)***	0					
Visit 2 (b) ***(MRI)***	(+/- 3)					
Visit 3 ***(Progress visit)***	4					
Visit 4 ***(Progress visit)***	+13					
Visit 5 ***(Progress visit)***	+17					
Visit 6 (a) ***(Final visit)***	+18					
Visit 6 (b) ***(Final MRI)***	(+/- 3)					

Participant ID			Initials		

VISIT 1 Screening Visit

INFORMED CONSENT										
Has the subject given written informed consent?	YES		NO							
If YES, date of consent			–	m	m	–	y	y	y	y

INCLUSION CRITERIA			
The following items MUST be answered YES for participant to be included in the trial		**YES**	**NO**
1	Participant is willing and able to give informed consent		
2	Aged 18 to 80 years old		
3	Participant diagnosed with type 2 diabetes mellitus based on the current American Diabetes Association guidelines.		
4	Echocardiographic LV hypertrophy (defined as an LV mass index of >115g/m2 for men and >95g/m2 for women indexed to body surface area or > 44g/m2.7 for women and >48g/m2.7 indexed to height2.7)		
5	Women of child bearing potential must agree to scheduled pregnancy testing prior to and during study treatment period and to use an appropriate method of contraception if sexually active.		

EXCLUSION CRITERIA			
The following criteria MUST be answered NO for participant to be included in the trial		**YES**	**NO**
1	Participants with type 1 diabetes mellitus		
2	Participants who have previously had an episode of diabetic ketoacidosis		
3	HbA1c < 48mmol/mol or >85mmol/mol (last known result within the last 6 months)		
4	Past or current treatment with SGLT2 inhibitor		
5	Allergy to any SGLT2 inhibitor or lactose or galactose intolerance		

6	BP ≥ 145/90mmHg		
7	Diagnosis of clinical heart failure		
8	LV systolic dysfunction (LVEF <45%) (last known result within in the previous 6 months)		
9	eGFR <45 ml/min (Last result within the previous month)		
10	Known liver function tests >3 times upper limit of normal (based on last measures and documented laboratory measurement in the previous 6 months)		
11	Serum sodium and potassium results out with the normal range		
12	Contraindications to cardiac MRI (e.g. claustrophobia, metal implants, penetrative eye injury or exposure to metal fragments in eye requiring medical attention)		
13	Body mass index < 23kg/m^2		
14	Body weight >150kg (Unable to fit in MRI scanner)		
15	Current treatment with a loop diuretic		
16	Currently receiving long term (>30 consecutive days) treatment with an oral steroid		
17	Any condition that in the opinion of the investigator may render the participant unable to complete the trial including non CV disease (e.g. active malignancy).		
18	History of human immunodeficiency virus		
19	Pregnant or breast feeding participants		
20	In a clinical trial of an investigational medical product within last 30 days		
21	Involvement in the planning and/or conduct of the trial (applies to Astra Zeneca or representative staff and/or staff at the trial site).		
22	Individuals considered at risk for poor protocol or medication compliance		

If any inclusion criteria is answered NO, or any exclusion criteria answered YES the participant is NOT eligible for the trial and must not be included in the study. If ineligible please document reason (s)

Signed		**Name**		**Date**	

VISIT - 1

Patient Characteristics
Source of recruitment_____
Age_____ years

Sex: 1. Male ☐ 2. Female ☐

SIMD 2012 Quintile_____
SMD 2012 Decile _____

PAST MEDICAL HISTORY	Yes	No
1. Ischaemic Heart Disease (Angina / MI)		
2. Dyslipidaemia		
3. Hypertension		
4. Stroke/TIA		
5. Atrial Fibrillation		
6. Chronic Obstructive Pulmonary Disease		
7. Chronic Kidney Disease		
8. Peripheral arterial disease		
9. Other – If yes specify		

Duration of diabetes_____ years

Angina or heart attack in a 1st degree relative < 60 years old?	Yes ☐	No ☐

CONCOMITANT MEDICATION (log updated)	Yes ☐	No ☐

SOCIAL HISTORY
Smoking status: Current smoker ☐ Never ☐ Ex- Smoker ☐
Average weekly alcohol intake: ☐☐☐ Units

Height (M)		Weight (Kg)	

EXAMINATION
NORMAL ☐ ABNORMAL ☐
Comments:

(Same arm should be used throughout trial – Average of three

Blood Pressure				
Arm	**Systolic (mmHg)**	**Diastolic (mmHg)**	**HR (BPM)**	**Temp (°C)**
1				
2				
3				
Average				
Ambulatory BP if borderline				

QRISK 2 2016 Score (%)	

	Yes	No	N/A
Female of child bearing potential?			
Urine pregnancy test positive?			

Electrocardiogram	**Yes** ☐	**No** ☐
Sinus Rhythm?	**Yes** ☐	**No** ☐
Rhythm if not Sinus_____		

SCREENING ECHOCARDIOGRAM **Date:**
LV Systolic function: 1. Normal/Mild ☐ 2. Moderate/Severe ☐
Severe Aortic stenosis: Yes ☐ No ☐
LV mass index of >115g/m2 for men or >95g/m2 for women indexed to BSA Yes ☐ No ☐
OR
LV mass index of >48g/$m^{2.7}$ for men or > 44g/$m^{2.7}$ for women indexed to height$^{2.7}$ Yes ☐ No ☐
LV mass indexed to BSA_____g/m^2
LV mass indexed to height$^{2.7}$_____g/$m^{2.7}$
Any other significant findings _____

Diastolic function assessment

E:A ratio	
Mitral Valve Deceleration time (ms)	
Septal S wave (cm/s)	
Lateral S wave (cm/s)	
Septal e' (cm/s)	
Lateral e' (cm/s)	
Lateral E:e'	
Septal E:e'	
Average E:e'	
Grade of diastolic dysfunction	
Global longitudinal Strain	

Venepuncture (Safety/Screening Bloods)	**Yes** ☐	**No** ☐
Samples required: • 2 x EDTA (purple) – FBC,HbA1c • 1 x clotted (golden) – U&E's, LFT's, cholesterol, HDL-cholesterol • 1x glucose (grey) – fasting glucose		

Administration	Yes	No
Patient given copy of consent		
Screening log updated		
Letter sent to GP to inform of screening/enrolment in trial		
Trial sticker and divider in notes		
Copy of consent, PIS and GP letter filed in medical notes		
Record of visit documented in medical notes		
CMRI safety checklist completed and sent to MRI (+ Copy of consent)		
Visit 2 booked and recorded on front of CRF		

Comments:

Signed		**Name**		**Date**	

Data transcribed in to DAPA-LVH Excel sheet;

Date:_____ **Signature:**_____ **Name Printed:**_____

Participant ID		

Initials		

VISIT – 2 (Randomisation)

PRE VISIT 2 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 1 baseline/safety bloods reviewed and recorded in bloods Log	☐

ID Confirmed	**Yes** ☐	**No** ☐
Consent Continued	**Yes** ☐	**No** ☐
CONCOMITANT MEDICATION (log updated)	**Yes** ☐	**No** ☐
Progress logs updated / Diabetic and Antihypertensive medication reviewed	**Yes** ☐	**No** ☐
AE/SAE (log updated)	**Yes** ☐	**No** ☐

Weight (Kg)	

(Same arm should be used throughout trial)

Average Blood Pressure (Arm)	Systolic (mmHg)	Diastolic (mmHg)	HR (BPM)
24hr Ambulatory Blood Pressure (Average)			
Ambulatory Blood Pressure (Daytime)			
Ambulatory Blood Pressure (Nocturnal)			

Waist Circumference (Cm)	
Hip Circumference (Cm)	

	Yes	No	N/A
Female of child bearing potential?			
Urine pregnancy test positive?			

Venepuncture (Safety/Screening Bloods) **Yes** ☐ **No** ☐

Samples required:

- 2 x EDTA (purple) – FBC,HbA1c
- 1 x clotted (golden) – U&E's, LFT's, cholesterol, HDL-cholesterol
- 1x glucose (grey) – fasting glucose

Venepuncture (Research Bloods) **Yes** ☐ **No** ☐

Samples required:

- 1x clotted (golden)
- 1x EDTA (purple)

Blood samples taken for storage (genetic analysis) **Yes** ☐ **No** ☐

Are ALL the inclusion/exclusion criterion met? – see screening visit. YES ☐ NO ☐

Is the participant eligible to take part in the Clinical Trial? YES ☐ NO ☐

Investigator's Signature: _____

Investigator's Name: _____

Date of signature: D D – M M – Y Y Y Y

RANDOMISATION

Participant ID			Initials		

Randomisation and log completed		YES			NO		

Study Medication Dispensed 10mg Dapagliflozin/Placebo	**Yes** ☐	**No** ☐
Date study medication to commence if MRI not performed : _____		

Has the subject had their Cardiac MRI	**Yes** ☐	**No** ☐
If no is date of CMR arranged	**NA** ☐ **Yes** ☐	**No** ☐

	Y	**N**
Patient given hypoglycaemia action leaflet		
Patient given Diabetic Ketoacidosis leaflet		
Checked patient's understanding of hypoglycaemia and diabetic ketoacidosis		

ADMINISTRATION	YES	NO
Record of visit in medical notes	☐	☐
Date of visit 3 booked and recorded on the front of CRF	☐	☐

Signed		**Name**		**Date**	

Data transcribed in to DAPA-LVH Excel sheet;

Date: _____ **Signature:** _____ **Name Printed:** _____

VISIT - 3

PRE VISIT 3 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 2 baseline/safety bloods reviewed and recorded in bloods Log	☐

ID Confirmed	**Yes** ☐	**No** ☐
Consent Continued	**Yes** ☐	**No** ☐
Concomitant medication (log updated)	**Yes** ☐	**No** ☐
Progress logs updated / Diabetic and Antihypertensive medication reviewed	**Yes** ☐	**No** ☐
AE/SAE (log updated)	**Yes** ☐	**No** ☐

Weight (Kg)	

Average Blood Pressure (Arm)	Systolic	Diastolic	HR

Waist Circumference (Cm)	
Hip Circumference (Cm)	

	Yes	No	N/A
Female of child bearing potential?			
Urine pregnancy test positive?			

Venepuncture (Safety/Screening Bloods)	**Yes**	☐	**No**	☐
Venepuncture (Research Bloods)	**Yes**	☐	**No**	☐
Date of fourth visit booked and recorded on front of CRF	**Yes**	☐	**No**	☐
Study Medication Dispensed 10mg Dapagliflozin/Placebo	**Yes**	☐	**No**	☐
Record of visit in medical notes	**Yes**	☐	**No**	☐

Signed		**Name**		**Date**	

Data transcribed in to DAPA-LVH Excel sheet;

Date:_____ Signature:_____ Name Printed:_____

VISIT - 4

PRE VISIT 4 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 3 baseline/safety bloods reviewed and recorded in bloods Log	☐

ID Confirmed	**Yes** ☐	**No** ☐
Consent Continued	**Yes** ☐	**No** ☐
Concomitant medication (log updated)	**Yes** ☐	**No** ☐
Progress logs updated / Diabetic and Antihypertensive medication reviewed	**Yes** ☐	**No** ☐
AE/SAE (log updated)	**Yes** ☐	**No** ☐

Weight (Kg)	

Average Blood Pressure (Arm)	Systolic	Diastolic	HR

Waist Circumference (Cm)	
Hip Circumference (Cm)	

	Yes	No	N/A
Female of child bearing potential?			
Urine pregnancy test positive?			

Venepuncture (Safety/Screening Bloods)	**Yes**	☐	**No**	☐
Venepuncture (Research Bloods)	**Yes**	☐	**No**	☐
Date of fifth visit booked and recorded on front of CRF	**Yes**	☐	**No**	☐
Study Medication Dispensed 10mg Dapagliflozin/Placebo	**Yes**	☐	**No**	☐
Record of visit in medical notes	**Yes**	☐	**No**	☐

Signed		**Name**		**Date**	

Data transcribed in to DAPA-LVH Excel sheet;

Date:_____ Signature:_____ Name Printed:_____

VISIT - 5

PRE VISIT 5 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 4 baseline/safety bloods reviewed and recorded in bloods Log	☐

ID Confirmed	**Yes** ☐	**No** ☐
Consent Continued	**Yes** ☐	**No** ☐
Concomitant medication (log updated)	**Yes** ☐	**No** ☐
Progress logs updated / Diabetic and Antihypertensive medication reviewed	**Yes** ☐	**No** ☐
AE/SAE (log updated)	**Yes** ☐	**No** ☐

Weight (Kg)	

Average Blood Pressure (Arm)	Systolic	Diastolic	HR

Waist Circumference (Cm)	
Hip Circumference (Cm)	

	Yes	No	N/A
Female of child bearing potential?			
Urine pregnancy test positive?			

Venepuncture (Safety/Screening Bloods)	**Yes**	☐	**No**	☐
Venepuncture (Research Bloods)	**Yes**	☐	**No**	☐
Date of sixth visit booked and recorded on front of CRF	**Yes**	☐	**No**	☐
Study Medication Dispensed 10mg Dapagliflozin/Placebo	**Yes**	☐	**No**	☐
Record of visit in medical notes	**Yes**	☐	**No**	☐

Signed		**Name**		**Date**	

Data transcribed in to DAPA-LVH Excel sheet;

Date: _____ Signature: _____ Name Printed: _____

Participant ID			Initials		

VISIT – 6

PRE VISIT 6 PHONE CALL	
Confirm patient appointment time/date/transport	☐
Visit 5 baseline/safety bloods reviewed and recorded in bloods Log	☐

ID Confirmed	**Yes** ☐	**No** ☐
Consent Continued	**Yes** ☐	**No** ☐
Concomitant medication (log updated)	**Yes** ☐	**No** ☐
Progress logs updated / Diabetic and Antihypertensive medication reviewed	**Yes** ☐	**No** ☐
AE/SAE (log updated)	**Yes** ☐	**No** ☐

Weight (Kg)	

Average Blood Pressure (Arm)	Systolic	Diastolic	HR
Ambulatory Blood Pressure (Average)			
Ambulatory Blood Pressure (Daytime)			
Ambulatory Blood Pressure (Nocturnal)			

Waist Circumference (Cm)	
Hip Circumference (Cm)	

Final Echocardiogram	**Date:**
LV Systolic function: 1. Normal/Mild ☐ 2. Moderate/Severe ☐	
Severe Aortic stenosis: Yes ☐ No ☐	
LV mass indexed to BSA_____g/m^2	
LV mass indexed to height$^{2.7}$ _____ g/m$^{2.7}$	
Any other significant findings _____	

Diastolic function assessment

E:A ratio	
Mitral Valve Deceleration time (ms)	
Septal S wave (cm/s)	
Lateral S wave (cm/s)	
Septal e' (cm/s)	
Lateral e' (cm/s)	
Lateral E:e'	
Septal E:e'	
Average E:e'	
Grade of diastolic dysfunction	
Global longitudinal Strain	

	Yes	No	N/A
Female of child bearing potential who is sexually active?			
Urine pregnancy test positive?			

Venepuncture (Safety/Screening Bloods)	**Yes**	☐	**No**	☐
Venepuncture (Research Bloods)	**Yes**	☐	**No**	☐
Record of visit in medical notes	**Yes**	☐	**No**	☐

Has the participant had their final Cardiac MRI		**Yes**	☐	**No**	☐
If no is date of CMR arranged	**NA**	**Yes**	☐	**No**	☐

End of study form completed			**Yes**	☐	**No** ☐

Signed		**Name**		**Date**	

Data transcribed in to DAPA-LVH Excel sheet;

Date:_____ **Signature:**_____ **Name Printed:**_____

DAPA-LVH Participant ID: _____ Initials: _____ Date: _____

Completion	**Y**	**N**
Did the subject complete the study?	☐	☐

Date of completion/withdrawal_____/_____/_____
Date of last dose of IMP_____/_____/_____

***If subject did not complete, what was the main reason (tick one):**	**DETAILS:**
Failed Eligibility (Screen fail) ☐	N/A
Advice from GP/ other healthcare professional ☐	
Adverse event ☐	
Participant's choice ☐	
On advice of investigator ☐	
Died ☐	N/A
Other ☐	

Follow-up

Is any follow-up required? ☐ ☐

If so, provide details:_____

_____

_____

Protocol

DAPA-LVH Case Report Form: Version 3.0: 21 Feb 2018 **PROTOCOL ID** 2015DM07

Where there any deviations from the protocol? ☐ ☐

See Protocol Deviations Log

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I verify that all the data contained in the CRF is complete and accurate.

Chief Investigator / Principal Investigator

Signed		**Name**		**Date**	

Baseline/Safety Blood + Pregnancy Log

	Visit 1 (Screening)	***Visit 2 (Baseline)***	***Visit 3 (4 weeks)***	***8 Weeks***	***12 weeks***	***16 weeks***	***Visit 4 (17 wee***	***21 weeks***	***25 weeks***	***29 weeks***	***33 weeks***	***Visit 3 (34 weeks)***	***38 weeks***	***42 weeks***	***46 weeks***	***50 weeks***	***Visit 6 (Final)***
Pregnancy Test																	

Lab Measurements	***Visit 1 (Screening)***	***Visit 2 (Baseline)***	***Visit 3 (Progress)***	***Visit 4 (Progress)***	***Visit 5 (Progress)***	***Visit 6 (Final)***
Full Blood Count (Date)						
Hb (g/L)						
HCT (%)						
WBC (x10^9/L)						
PLT (x10^9/L)						
Liver Function Tests (Date)						
ALT (U/L)						
BILIRUBIN (umol/L)						
ALKALINE PHOSPHATASE (U/L)						
ALBUMIN (g/L)						

Urea and Electrolytes (Date)						
Sodium (mmol/L)						
Potassium (mmol/L)						
Urea (mmol/L)						
Creatinine (umol/L)						
GFR (mL/min)						
Glucose and Lipids (Date)						
Fasting glucose (mmol/l)						
HbA1c (mmol/mol)						
Total Cholesterol (mmol/l)						
HDL-Cholesterol (mmol/l)						
TC/HDL ratio						
LDL-Cholesterol (mmol/l)						
TG (mmol/l)						

PROGRESS LOG

Change in parameter	***Visit 1 (Screening)***	***Visit 2 (Baseline)***	***Visit 3 (Progress)***	***Visit 4 (Progress)***	***Visit 5 (Progress)***	***Visit 6 End***
Medications	***√= ON MED ↓= Dose Reduction ↑= Dose Increase Free Text Details***					
Insulin						
Metformin						
Sulphonylureas						
DPP-IV / GLP-1 Ag						
ACE/ARB						
Calcium Channel Blockers						
Thiazide diuretics						
Other Anti Hypertensive agents						
Others						

Participant ID		

Initials		

Adverse Event Log		
Study title DAPA-LVH Does Dapagliflozin Regress Left Ventricular Hypertrophy in Patients With Type 2 Diabetes?		
REC ID: 16/ES/0131	**Sponsor R&D Protocol ID: 2015DM07**	**EudraCT ID: 2016-000715-33**
Sponsor: University of Dundee – NHS Tayside	**Chief Investigator: Prof Allan Struthers**	**Principal Investigator: Alex Brown**
Site: Tayside	**Participant ID:**	

Description of adverse event (provide additional information on notes pages if required)	**Date of onset**	**Date reported to Investigator/ team**	**Severity** 1. Mild 2. Moderate 3. Severe	**Causality** 1. Unrelated 2. Possible 3. Probable 4. Definite	**Action taken – please list all that apply** 1. None 2. Hospitalisation 3. Intervention stopped 4.Con Meds commenced (record on Con Meds Log) 5. Other (specify)	**Outcome** 1. Recovered 2. Recovered with sequalae 3. Ongoing 4. Disability or incapacity 5. Death 6. Unknown	**Is this a Serious AE?** **YES* or NO**	**Is this an Adverse reaction?** **YES or NO.** **If yes, report using yellow card scheme**	**Date resolved** (Enter date resolved/date of death or tick if still ongoing 30 days after last study visit. SAEs should be followed up until resolved)	**Signature and Date**
									*complete an SAE form and email to the Sponsor pharmacovigilance.tayside@nhs.net	
	__/__/__	__/__/__							__/__/__ or ☐	
	__/__/__	__/__/__							__/__/__ or ☐	
	__/__/__	__/__/__							__/__/__ or ☐	

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	__/__/__	__/__/__							__/__/__ or ☐	

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Medication Log

Medication	*Code*	*Ongoing at start or start date (if known)*	*Ongoing at end or stop date (if stopped)*
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			

Medication Coding:

1. ACEI	**7. MRAs**	**13. DDP-IV Inhibitors**
2. ARBS	**8. Asprin**	**14. GLP-1 agonists**
3. Ca Channel Blockers	**9. Clopidogrel**	**15. Thiazolidinedione**
4. Thiazide Diuretic	**10. Statins**	**16. Insulin**
5. β Blocker	**11. Metformin**	**17. Loop diuretic**
6. α Blocker	**12. Sulphonylurea**	**18. Other**